Agglutination of Bladder Cells by Concanavalin A during the Early Phase of Treatment of Rats with N-Butyl-N-(4-hydroxybutyl)nitrosamine

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ABSTRACT

N-Butyl-N-(4-hydroxybutyl)nitrosamine was given to male Wistar rats at a dose of 0.05% in the drinking water for one to five weeks, and agglutination of cells isolated from their bladder by concanavalin A (Con A) was determined at intervals during and after treatment. Mucosal cells were isolated from everted bladder by ethylenediaminetetraacetate treatment and sonication. As early as one week after the start of treatment, Con A agglutination of bladder cells became apparent. Two weeks after the end of treatment, most of the treated animals showed agglutinability of isolated bladder cells with Con A. Measurement of agglutinability of isolated bladder cells with Con A might be a useful way of detecting very early changes in bladder carcinogenesis.

INTRODUCTION

The main problems in the treatment of human superficial bladder cancer are the multicentric development of tumors and the high frequency of recurrence after transurethral resection (1, 4). To solve these problems, more information is required on the biological characteristics of transitional epithelial cells during carcinogenesis.

The plant lectin Con A agglutinates and kills transformed cells, but it has little effect on normal cells (6, 7, 19, 22, 23, 25).

The mucosal surface of normal rat urinary bladder is lined by 3 layers of cells: large flat superficial polypoid cells; intermediate cells; and basal cells (24, 26). BBN is a specific bladder carcinogen in rats (3, 9, 15). On treatment with BBN or other carcinogens, such as N-methyl-N-nitrosourea, ESNS, or FANFT, for 2 to 6 weeks, the mucosal cells of rat bladder show various morphological changes, such as hyperplasia and the appearance of microvilli (2, 5, 8, 11). Some of the early changes are reported to be reversible if carcinogen treatment is discontinued, but after about 8 weeks of treatment most of the changes are irreversible and progress to bladder carcinogenesis.

In an attempt to detect early epithelial changes during carcinogenesis, we studied Con A agglutination of cells isolated from rat bladder during BBN treatment and 2 weeks after the end of treatment. We also examined the mucous lining of the urinary bladder of the rats by scanning electron microscopy and the usual light microscopy.

MATERIALS AND METHODS

Male Wistar rats weighing 140 to 160 g were used throughout. They were kept 5 to a cage and fed on CE-2 pellet diet (CLEA, Japan). BBN, purchased from Izumi Chemicals Co., Yokohama, Japan, was added to the drinking water as described previously (12). Eight groups of 5 animals were given 0.05% BBN for 3, 5, 7, 10, 14, 21, 28, and 35 days, respectively. After treatment, the animals were sacrificed; 4 were used for assay of agglutination with Con A and 1 was used for electron microscopic and light microscopic examination. To examine the reversibility of the early changes induced by BBN, 5 groups of 5 animals each were given 0.05% BBN for 7, 14, 21, 28, and 35 days, respectively, and then normal tap water for 2 weeks. Then 4 animals from each group were used for agglutination assay, and 1 was used for morphological examinations. Control animals were given tap water throughout and were sacrificed at the corresponding times for examination. As negative controls, 5 animals were given t-BBN, a noncarcinogenic, nonmutagenic analog of BBN (20), at a concentration of 0.05% in the drinking water for 1 week and then examined.

Cells were isolated from rat urinary bladder as reported previously (13). Briefly, the bladder was removed, everted, and incubated in 2.0 ml of 0.15 M NaCl containing 5 mM EDTA (pH 4.5). Then it was sonicated in an ultrasonic cuvet washer, and the cells isolated from 4 bladders were combined and collected by centrifugation. Agglutination assay was performed in a final volume of 40 µl of phosphate-buffered saline (8.0 g NaCl, 0.2 g KCl, 1.146 g Na2HPO4, and 0.2 g KH2PO4, in a total volume of 1000 ml, pH 7.4) containing 2 to 5 × 106 cells/ml and Con A, 200 or 400 µg/ml, with or without a-methylmannoside, 10 µg/ml. After gentle shaking on a micromixer for 30 min at 37°C, the number of cell aggregates consisting of more than 3 cells per 200 free or aggregated cells was counted in a hemocytometer (16). Con A and a-methylmannoside were obtained from Sigma Chemical Co., St. Louis, Mo.

The dye exclusion test with trypan blue was used to determine the viability of isolated cells.

The isolated cells were examined by light microscopy after staining with Papanicolaou stain. The respective numbers of superficial cells, which are characteristically large, flat, and frequently binucleate cells, and of smaller intermediate or basal cells were counted.

The morphology of the mucosal lining of rat bladder was...
examined by scanning electron microscopy and light microscopy (13) as described previously.

RESULTS

The time-dependent change in agglutination of isolated bladder cells by Con A immediately after BBN treatment for various periods is shown in Chart 1. Even 5 days after the start of treatment, Con A agglutination of isolated cells was already detectable and by the seventh day, it was marked. The number of cell aggregates was not proportional to the concentration of Con A, but 400 μg of Con A caused more agglutination than 200 μg of Con A. By Day 21 of treatment, agglutination appeared to have reached an almost constant value with both concentrations of Con A. Throughout the experiment, cell agglutination by Con A was specifically inhibited by α-methylmannoside. The large superficial cells were not agglutinated by Con A, and the cell clumps consisted of medium-sized and small cells. Differential counts of isolated cells showed that the number of large cells decreased immediately after the start of BBN treatment and remained low during the rest of the experimental period; normally, 10 to 15% of the isolated cells were large (13, 17), but during treatment they decreased to under 5% of the total. Scanning electron microscopy of bladder mucosa in situ showed that microvilli or a network of rounded microridges appeared on the luminal surface during treatment (Figs. 1 and 2). From 7 days after the beginning of BBN treatment, the cells on the surface of the bladder epithelium varied greatly in size, and most of them had either rounded or leafy microridges on their luminal surface. Intermediate forms between rounded microridges and microvilli were also observed. The luminal surface of control bladder was covered with uniform polygonal superficial cells, which had fine microridges but no microvilli.

Results on agglutination by Con A 2 weeks after the end of BBN treatment for 1 to 5 weeks are shown in Chart 2. Agglutination by Con A did not decrease after the end of BBN treatment for even only 1 week but was almost constant and independent of the period of BBN treatment, being similar to the constant level observed immediately after BBN treatment for 21 to 35 days. Findings by electron microscopy were basically similar to those obtained immediately after BBN treatment for 21 to 35 days. Moreover, although differential counts of the isolated bladder cells revealed recovery of the number of large cells to about 10% of the total, many of these large cells had only one large nucleus and differed in size and shape from normal large binucleate superficial cells.

In dye exclusion tests, more than 95% of the isolated cells took up trypan blue, the percentages being similar in BBN-treated and untreated cells. No agglutination was observed after treatment with t-BBN. The numbers of cell aggregates obtained after treatment with t-BBN for 1 week were 5 of 200 cells with both 200 and 400 μg of Con A.

DISCUSSION

In this work, we examined early changes of mucosal cells of rat bladder during and after treatment with BBN by measuring the agglutination of isolated bladder cells with Con A. Bladder cells isolated by ultrasonication were agglutinated by Con A even after BBN treatment for only 5 days. During longer treatment, agglutination increased with time and appeared to reach a constant level from the 21st day. Our important findings were that Con A agglutination of bladder cells induced by BBN was irreversible even by 2 weeks after the end of BBN administration and that this method could detect specific early changes of bladder cells that were undetectable by light microscopy. Electron microscopy, however, showed that during BBN treatment microvilli and networks of rounded or leafy microridges appeared on the luminal surface of the bladder in situ. Isolation of the cells by sonication seemed to damage them appreciably, because most of the isolated cells took up trypan blue. However, changes of the membrane induced by BBN treatment could be detected as cell agglutination by Con A.

There are reports of the development of microvilli and mild hyperplasia on treatment with FANFT or BBN for 2 to 4 weeks and their disappearance on discontinuation of carcinogen treatment (2, 8, 11). Treatment with FANFT or BBN for 8 weeks induced focal hyperplasia, which was regarded as an early preneoplastic change (2, 8, 11). The appearance of a glyco- calyx was also reported as a specific preneoplastic and neoplastic marker induced by N-methyl-N-nitrosourea, ESNS, or BBN (5). In our study, however, cell agglutination by Con A did not regress after the end of BBN administration.

There are also reports that dividing cells resemble transformed cells in that they are covered with microvilli and agglutinated with Con A (22, 27). However, we could not find any mitotic figures in the mucosal lining of the bladder at any time during or after short-term treatment with BBN. Therefore, the
increase of cell agglutination by Con A detected in this study does not seem to be a result of mitosis. Recently, Pietras (21) reported increased cathepsin B activity and alterations of the membranes of cells isolated from bullfrog or rabbit urinary bladder when the cells were treated with dibutyl nitrosamine in vitro. Thus, proteases may be involved in exfoliation of superficial cells and changes of membrane properties during the early stage of bladder carcinogenesis.

At present, we think that the mechanism of cell agglutination by Con A may be as follows. Administration of BBN induces the exfoliation of the large superficial cells and appearance at week treatment with 0.05% BBN were, respectively, 55, 9, and 100% after 20 weeks and 100, 73, and 18% after 40 weeks, and the results of in vitro experiments on Con A agglutination in the bladder epithelium of different animal species. Cancer Res., 36: 2526—2531, 1976.

Fig. 1. Scanning electron micrograph of the luminal surface of the bladder in situ after treatment with BBN for 14 days. Short microvilli and rounded microridges are shown. x, 2500.

Fig. 2. Scanning electron micrograph of the luminal surface of normal rat bladder. The polygonal faces of superficial cells are covered with fine microvilli. Cell boundaries are distinct. x, 1500.

Agglutination of BBN-treated Bladder Cells by Con A


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