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Prophylaxis of FANFT Induced Bladder Tumours in Rats by Intravesical DNCB Instillation

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Summary. Immunotherapy to prevent the appearance of superficial bladder tumours was studied in the (N(4-(5-Nitro-2-Furyl)-2-Thiazolyl Formamide) (FANFT) rat model. Intravesical 2,4-dinitro-1-chlorobenzene (DNCB) was instilled beginning at six weeks after feeding the animals FANFT and given at monthly intervals. Results showed no difference between control versus treated groups in tumour growth or lymphocytic infiltration near tumours. A significant incidence of calculi formation was noted in the DNCB group.

Key words: Bladder Cancer, FANFT, DNCB, Calculi.

The use of (N(4-(5-Nitro-2-Furyl)-2-Thiazolyl) Formamide) (FANFT) to induce transitional cell carcinoma in rats has been the subject of several reports (2, 3, 4, 9, 11). All animals develop bladder tumours between 24 and 36 weeks (2, 9, 11) with the first irreversible changes seen at eight weeks (9, 11). Several recent studies have used 2,4-dinitro-1-chlorobenzene (DNCB) to induce delayed hypersensitivity and attract lymphocytes to tumour areas. Immunotherapy based on the induction of delayed hypersensitivity response has produced remissions in vaginal cancer and skin cancers (5, 7, 8, 10). Based on the fact that immunotherapy results are best with small tumour load, this experiment was designed to start DNCB therapy as a preventative agent when the first microscopic bladder mucosal changes were occurring in animals fed a diet containing FANFT.

MATERIALS AND METHODS

Sixty female Sprague-Dawley rats averaging 200 grams were divided into two groups. Both groups were fed 0.2 percent FANFT (Saber Laboratories, Inc., Morton Grove, Illinois) in Purina Chow. All animals were given free access to water.

Dinitrochlorobenzene (DNCB), 100 μg/ml in 94 percent alcohol was diluted with normal saline to give a concentration of 100 μg/ml. Beginning at six weeks after feeding the animals FANFT, 50 μg DNCB (0.5 ml) was placed intravesically in the treated group at four week intervals. In a similar manner, 0.5 ml normal saline was placed intravesically in the control group.

Bladder catheterization was accomplished by inserting a No. 18 polyethylene intravenous catheter per urethram. A solution of 0.5 ml was then instilled for 30 min using a silk tie around the urethra to keep the solution in the bladder. Both control and DNCB treated animals were skin tested with 50 μg/0.1 ml DNCB intradermally. All animals tested had a positive skin reaction.

The animals were sacrificed at 24 weeks with the bladder fixed in 10% formalin. The specimens were stained with hematoxylin and eosin and read without prior knowledge whether the specimen was from the control or treated groups.

RESULTS

Forty-three of 60 animals were alive at the end of the experiment, 20 in the treated group and 23 controls. The specimens were evaluated for the presence of carcinoma, grade of tumour, mucosal changes and presence of lymphocytic infiltration. No difference between treated and control groups was found (Table 1). No difference in lymphocytic infiltration was noticed between treated and control groups.

Five animals in the DNCB treated group formed 11 stones - three staghorn, five ureteric and three in the bladder. Only two stones were seen in the control group - one staghorn and one bladder. All the animals with stones had developed

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Table 1.

	Control	DNCB
Normal	8	10
Squamous Metaplasia	0	6
Grade I Transitional	10	5
Grade II Transitional	2	2
	20	23

transitional cell carcinoma. Calculus formation has not been previously reported in the FANFT rat model or with DNCB intravesical therapy.

DISCUSSION

Starting immunotherapy at six weeks did not inhibit FANFT induced bladder cancer. Presensitisation with intradermal DNCB does not seem necessary since all animals tested in both groups had positive skin reaction. Sensitisation may begin with the first intravesical instillation of DNCB in the treated group or environmental factors may sensitise the animals.

Lymphocytic infiltration near bladder tumours has been felt to provide a good prognostic sign and indicate increased immune defences (1). No increase in lymphocytic infiltration was observed in our treated group in comparison to the control group. This may mean that the immune defences may not have been adequately stimulated by topical therapy. Intralesional injection of Bacillus Calmette-Guerin (BCG) has stimulated tumour regression while topical application has shown no response (6).

Intravesical DNCB instillation has been used before in this animal model with the intent of preventing bladder tumour (6). In that study, therapy was started twelve weeks after carcinogen induction. Our experiment was designed to begin immunotherapy at the time the first irreversible changes appeared in this animal model (6-8 weeks) since this is the point of minimal tumour load. No differences, however, were found among treated or control groups.

An unexpected finding was the appearance of multiple urinary calculi in numbers significantly higher in the DNCB treated animals. Studies are underway to identify the possible aetiology of these urinary stones.

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