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Histopathology of BCG and Thiotepa Treated Bladders

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Summary. In an effort to characterize the pathophysiological differences between the modes of action of BCG and thiotepa in the treatment of superficial bladder cancer, normal female rats received weekly intravesical instillations of both agents. The animals were sacrificed and their bladders were examined pathologically to determine if there were histological differences between the cellular infiltrates found in the BCG and thiotepa treated bladders. Mononuclear cells, particularly T-cells, predominated in the BCG treated bladders whereas polymorphonucleated cells predominated in the thiotepa treated bladders suggesting that there may be an immune aspect to the former therapy. The presence of T-cells following BCG therapy suggests a T-cell mediated immune response.

Key words: Intravesical chemotherapy, BCG, Thiotepa.

Introduction

Recent evidence [1–3] suggests that intravesical BCG may be a more effective agent in the prevention of recurrence of superficial bladder tumors than the currently standard agent, thiotepa [4]. Controversy exists as to the mode of action of these two agents. It is generally agreed that thiotepa exerts a direct cytotoxic effect on bladder mucosa and coexisting superficial bladder tumors [5, 6]. Whether BCG acts in a similar manner or exerts an essentially different immune effect has not been examined. The purpose of this research was to evaluate the histopathologic differences in bladder wall cellular infiltrates following the intravesical application of either BCG or thiotepa. In addition an effort was made to further define the mononuclear cell infiltrates.

Table 1. Schedule of animal bladder preparation

Weeks	No. of Bladders	
	BCG	Thiotepa
2	9	8
4	8	5
6	5	6
8	5	5
Total	27	24

Materials and Methods

Animals and Drugs. Fifty-one 3 month old female Copenhagen/Fisher rats weighing 150–200 g were employed; they were divided into two groups: Group I consisted of 27 animals that received BCG, and Group II consisted of 24 animals that received thiotepa (Table 1). BCG was Tice strain, (lot no. 105,134), 2.5×10^8 colony forming organism/ml diluted in 50 cc of saline. Thiotepa was manufactured by Lederle Inc. (lot no. 721,506), 30 mg diluted in 30 cc of saline.

Administration. The rats were anesthetized and the bladders were compressed to remove any preexisting urine. The perineum was cleansed and a no. 22 Deseret catheter was inserted and 0.5 ml of BCG or thiotepa was inserted. The urethra was gently ligated and the rat was placed on white paper and observed for two hours to make sure that the entire volume of the instilled agents remained. Catheters were reinserted and any residual chemotherapy was removed and the bladders were flushed with saline. This schedule was repeated weekly for eight weeks.

Histopathology. After the initiation of therapy animals from both groups were sacrificed at two weeks intervals, their bladders removed, flushed with saline, and divided into 2 portions. One of the portions was used for routine histology and the other portion was frozen for use later for immunoperoxidase staining. For routine histology bladder tissue was fixed, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Cellular infiltration into the mucosa and submucosa was determined to be either mononuclear or polymorphonuclear and each slide graded on a scale of 0 to +4.

Table 2. Cellular infiltration BCG treated bladders

Week	2		4		6		8	
Cells	Mono	Poly	Mono	Poly	Mono	Poly	Mono	Poly
Animal # 1	0	0	2	0	4	1	2	3
2	1	0	2	1	2	0	1	3
3	1	0	1	0	3	2	1	2
4	1	0	2	0	3	5	3	2
5	5	0	3	1	3	0	3	2
6	1	0	2	0				
7	0	0	2	1				
8	0	0	1	1				
9	0	0						
M	0.56	0.0	1.88	0.50	3.00	0.70	2.00	2.40
SD	±0.50	±0.0	±0.64	±0.53	±0.71	±0.84	±1.00	±0.55

Table 3. Cellular infiltration thiotepa treated bladders

Week	2		4		6		8	
Cells	Mono	Poly	Mono	Poly	Mono	Poly	Mono	Poly
Animal # 1	1	1	1	3	4	1	2	3
2	0	1	1	4	0	4	1	2
3	0	0	1	3	2	4	0	1
4	1	1	1	4	1	3	1	3
5	1	1	2	2	1	3	2	2
6	1	0			0	0		
7	1	0						
8	1	0						
M	0.63	0.38	1.20	3.20	0.83	2.50	1.20	2.20
SD	±0.52	±0.52	±0.45	±0.84	±0.75	±1.64	±0.84	±0.84

Table 4. Mononuclear infiltrate

Therapy	Week 2	Week 4	Week 6	Week 8
BCG	0.56 ± 0.50	1.88 ± 0.64	3.0 ± 0.71	2.0 ± 1.0
(No.)	(9)	(8)	(5)	(5)
Thiotepa	0.63 ± 0.52	1.20 ± 0.45	0.83 ± 0.75	1.2 ± 0.84
(No.)	(8)	(5)	(6)	(5)
P	NS	NS	> 0.01	NS

Mononuclear Cell Characterization. T-cells in tissue sections were identified by the use of a mouse anti-rat T-cell [7, 8] monoclonal antibody (W3/13) (Pel-Freeze Biologicals). Cryostat sections of 6–10 μm thickness were air dried, then fixed in acetone for 10 min. Sections were washed in 0.01 m phosphate-buffered saline, ph 7.4, then covered with the antibody. After washing with PBS, slides were incubated with a peroxidase conjugated, goat anti-mouse IgG (Miles) for 30 min, and stained for peroxidase activity with 3,3-diaminobenzidine – tetrahydrochloride (DAB, Sigma).

Results

The BCG treated bladders had a mean mononuclear cell infiltration grade of 0.56, 1.88, 3.00 and 2.00 for weeks 2, 4, 6, and 8 a polymorphonuclear cell infiltrate grade of 0.0, 0.50, 0.70, and 2.40 for weeks 2, 4, 6, and 8 respectively (Table 2). The thiotepa treated bladders had a mean mononuclear cell infiltrate grade of 0.63, 1.20, 0.83, and 1.20 for

Table 5. Poly-morphonuclear infiltration

Therapy	Week 2	Week 4	Week 6	Week 8
BCG	0.0 ± 0.0	0.50 ± 0.53	0.70 ± 0.84	2.40 ± 0.55
(No.)	(9)	(8)	(5)	(5)
Thiotepa	0.38 ± 0.52	3.20 ± 0.84	2.50 ± 1.64	2.20 ± 0.84
(No.)	(8)	(5)	(6)	(5)
P	NS	> 0.01	> 0.05	NS

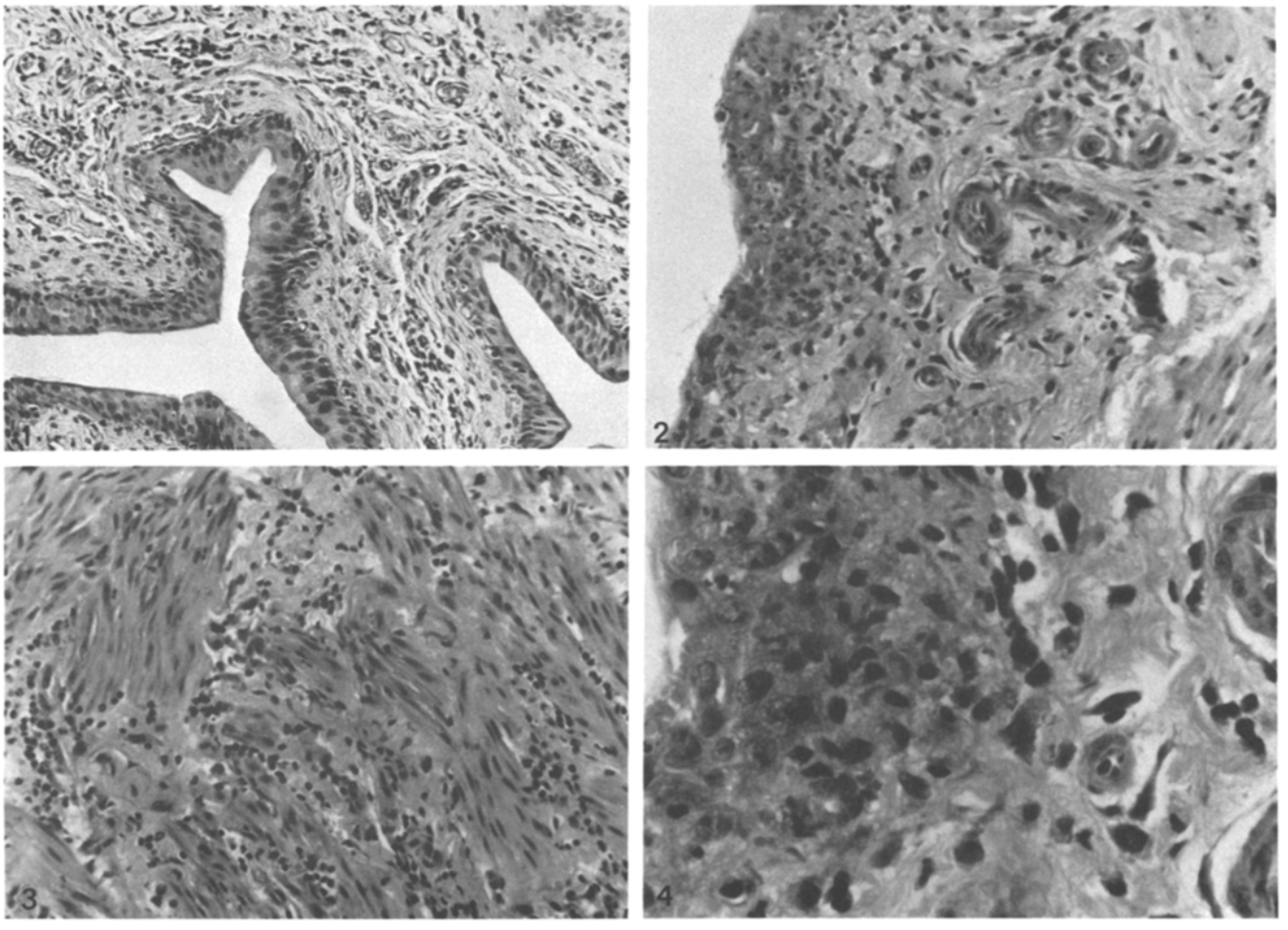

Fig. 1. Urinary bladder after two weeks of BCG therapy (H&E x 120)

Fig. 2. Urinary bladder after two weeks of thiotepa therapy (H&E x 120)

Fig. 3. After 4 weeks of thiotepa therapy (H&E x 120). Note PMN infiltration

Fig. 4. After 8 weeks of BCG change to BCG therapy (H&E x 250). Note mononuclear as well as PMN infiltration

weeks 2, 4, 6, and 8, and a polymorphonuclear cell infiltration grade of 0.38, 3.20, 2.50, and 2.20 for weeks 2, 4, 6, and 8 respectively (Table 3).

There was a statistically significantly greater mononuclear cell infiltrate at week six in the BCG treated bladders compared to the thiotepa treated bladders (Table 4). There was a statistically significantly greater polymorphonuclear cell infiltrate at weeks four and six in the thiotepa treated bladders (Table 5).

There was a relatively mild cellular response at two weeks in both the BCG (Fig. 1) as well as the thiotepa (Fig. 2) treated bladders. The BCG mononuclear cellular response peaked at six weeks and was intense. The thiotepa polymorphonuclear response peaked at four weeks (Fig. 3). By

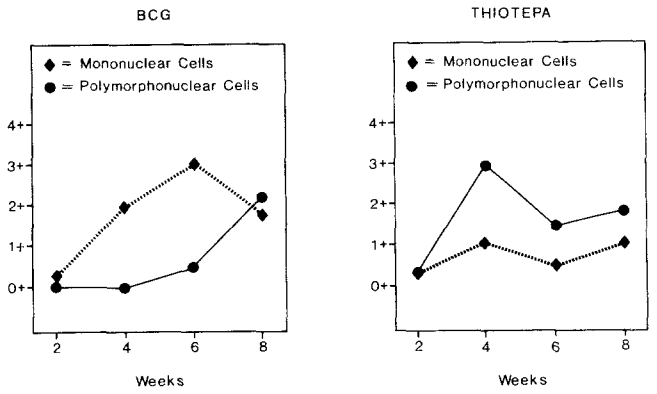

Fig. 5. Cellular infiltration (grade of infiltration)

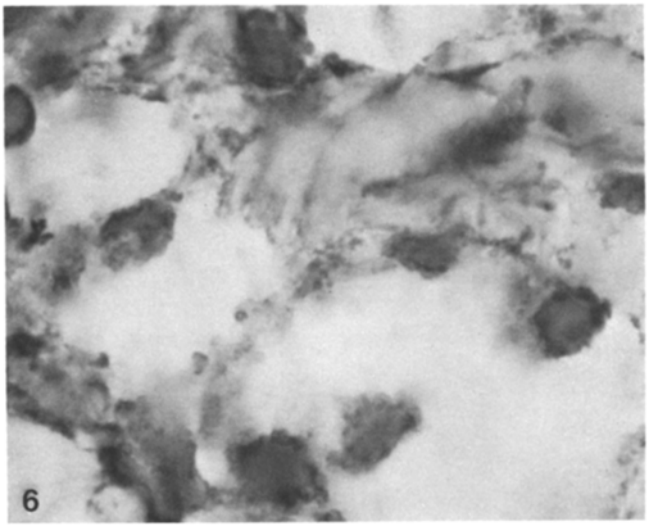

Fig. 6. x 520 magnification. At this higher magnification, note the prominent T-cell infiltration

week eight there was a mixed mononuclear and polymorphonuclear response in both the BCG and thiotepa treated bladders (Fig. 4). In summary, there was a preponderantly mononuclear cell infiltrate in the BCG treated bladder at weeks four and six and a preponderantly PMN infiltration in the thiotepa treated bladders at weeks four and six (Fig. 5).

Mononuclear cells were determined to be T-lymphocytes (Fig. 6).

Discussion

The immunotherapy of cancer is of only theoretical interest except in the treatment of bladder cancer where it appears effective [9–13]. Recent reports suggest that intravesical BCG may be more effective in the treatment of superficial bladder tumors than the currently standard agent, thiotepa. Controversy exists as to whether the favorable response to BCG is immunologically mediated or whether it is secondary to a chemical or irritative and inflammatory response.

Bacillus Calmette-Guerin (BCG) is an attenuated Mycobacterium bovis [14]. Its use in cancer patients was prompted by observations that tuberculosis patients had less cancer than individuals without TB [15] and that BCG innoculated individuals had a lower incidence of leukemia [16]. BCG influences numerous immunologic reactions: humoral immunity [17], antigen dependent cellular cytotoxicity [18], cell-mediated immunity [19, 20] lymphocyte activity [21], macrophage function [22], NK cells [23, 24], and stem cell production [25]. It is theorized that these influences are, in general, immunostimulating and therefore beneficial.

If antitumor activity is desired, the intralesional administration of BCG is most effective [26]. Of particular significance was the observation of regression of a non-injected lesion simultaneous with the disappearance of a BCG injection lesion in the same animal [27]. BCG has been therapeutically effective when delivered intravenously [28], intrapleurally [29], intraperitoneally and intravesically.

While BCG intravesical therapy for superficial bladder tumors is gaining wider clinical acceptance relatively few experimental investigations have been undertaken to determine the mechanism of action of BCG in this setting. Albert injected BCG directly into mouse bladders and noted immunostimulation as well as a systemic humoral response [30]. Lamm demonstrated the presence of mononuclear cell infiltrates in human BCG treated bladder walls for as long as six months post-therapy [31]; he also hypothesized that BCG stimulated lymphocytes and macrophages exert a cytotoxic effect on tumor cells. Brosman noted that for BCG therapy to be effective the bladder tumor patients must be responsive to the immunostimulant and Morales further observed that these patients should also be sensitized to the antigen [1]. Intravesical BCG can also give rise to circulating BCG antibodies which may participate in the immune response but which can also be utilized to monitor therapy [32, 33].

In this comparative study BCG treatment resulted in a sharp rise in the number of filtrating mononuclear cells up to week 6 and a gradual increase of PMN cells. Thiotepa treatment resulted in an initial infiltration of polymorphonuclear cells and a subsequent infiltration of mononuclear cells. Recent immunohistologic advances have made it possible to identify lymphocyte subpopulations and further evaluation of the mononuclear cell infiltrates revealed that they were primarily of T-cell origin. While this experiment was conducted in normal bladders it is assumed that similar findings would occur in tumor bearing bladders.

These findings support the concept that there is a fundamental pathopysiological difference between the modes of action of BCG and thiotepa in their effect against superficial bladder tumors. It is hypothesized that intravesical BCG is an immunostimulant. This preliminary investigation supports the contention that the BCG effects may, in part, be immunologically mediated. Experiments are underway to determine the phenotype of the infiltrating T-cells.

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