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Effects of intravesical *Corynebacterium parvum* on recurrences of superficial tumors of the urinary bladder

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We studied 96 patients with superficial tumors of the urinary bladder treated by transurethral resection in order to investigate the value of intravesical *Corynebacterium parvum* (CP) to prevent recurrences. In 52 cases, two vials (2 ml, 4 mg CP) diluted in 60 ml buffer saline were intravesically administered weekly 12 times and then monthly for a period of 2 years. All patients were studied in a 3 year follow-up by cytology, histology and endoscopy. Recurrences were observed in 21.1% of cases in the CP treated group and in 54.5% of cases in the untreated group. CP immunostimulation is less effective in preventing recurrences in patients with malignant cells as indicated by urine smears in the post-operative period. Morphological changes of the bladder wall due to CP administration in patients without recurrences are described. Chronic lymphocyte infiltrate appears to be an essential event for the action of CP as an adjuvant therapy in urinary bladder cancer.

Key words: Corynebacterium parvum (CP), intravesical immunotherapy, recurrence, superficial tumor, urinary bladder.

Introduction

Superficial tumors of the urinary bladder are characterized by their high rate of recurrences (in 43–86% of cases) and progression in 20–25% of cases.[1–3] Many attempts have been made to reduce recurrences using chemotherapeutic and immunostimulant agents by general or intravesical route. In 1976, Morales *et al.*[4] introduced adjuvant immunotherapy with intracavitary bacillus Calmette-Guerin (BCG) in the immediate post-operative period and reported good results in about 60% of cases. Some authors published very good results, especially in carcinoma *in situ* (CIS), reporting complete response in 48% of cases with a follow-up of 43.7–51.6 months.[5,6] The clinical value of intracavitary BCG is not completely established and about 10% of treated patients developed granulomatous lesions in the bladder, prostate, liver, lung or other organs.[7–9]

In view of results obtained with different strains of BCG and the relatively high rate of complications with its intracavitary administration, we investigated the clinical value and morphological modifications of the urinary bladder wall induced by intravesical *Corynebacterium parvum* (CP) immuno-stimulant as a possible adjuvant in the post-operative treatment of superficial tumors.

Materials and methods

We studied 96 patients with superficial tumors of the urinary bladder. All patients were treated by transurethral resection (TUR) and then 52 of them were treated intravesically with CP. From a histologic point of view there were four cases with CIS, 62 cases with Ta and 30 with T1 transitional cell carcinomas; the degree of differentiation was G1 in 73 casese and G2 in 23 cases. All patients were controlled in a follow-up by urine cytology monthly in the first year and three monthly over the next 2 years, and by cystoscopy at 3 months in the first year and at 6 months in the second year.

We first performed cytodiagnosis in each case 5 days after TUR; the urine samples were prepared for cytologic study using rapid blue polychrome tanin staining.[10] The technique and results of urine smears have been published by us elsewhere.[11] Biopsies from the initial lesions and recurrences were taken at the moment of intervention and from apparently normal mucosa in cases without recurrences at 6 and 24 months. All slides were stained with van Gieson's and hematoxylin–eosin methods. The presence or absence of malignant cells on urine smears, the histological form and the

Table 1. Post-operative cytology in 96 patients with superficial tumors of the bladder

'T'/cytodiagnosis	Negative	Positive
CIS	1	3
Ta	54	8
Tl	25	5
Total	80 (83.3%)	16 (16.6%)

Table 2. Distribution of cases treated with CP and untreated cases using 'T' element

Cases/'T'	CIS	Ta	Tl	Total
CP treated cases	4	32	16	52
Without immunotherapy	—	30	14	44

degree of differentiation of transitional cell carcinomas were noted in each case. Sixteen out of 96 patients presented malignant cells at cytodiagnosis, their distribution is shown in Table 1.

After TUR, 52 of 96 cases were treated by intravesical CP beginning on the 10th day of the post-operative period (CP vaccine, made by CP-killed bacteria, Mérrieux strain, vials of 2 ml containing 2 mg/ml of dried residue, produced by 'Cantacuzino Institute', Bucharest). Distribution of cases treated by CP and untreated cases, using the 'T' element, is presented in Table 2. The technique of adjuvant immunotherapy with CP consists of intravesical administration of two vials of CP diluted in 60 ml of buffered saline, once a week for 12 weeks and then monthly for a 2 year period. In cases with recurrences during this period, the procedure was repeated and started with another series of 12 instillations. All patients were controlled in a follow-up by cytology, histology and endoscopy for the next 3 years.

Results

There were 35 patients with recurrences out of 96 we studied, 11 in the group treated by intravesical CP and 24 in the untreated group during the 3 years of survey. One case recurred twice and three cases recurred 3 times, the last recurrences belonged to the untreated group. Thirteen out of 35 cases with recurrences had malignant cells in voided urine at the first post-operative cytodiagnosis, all of them treated with CP. We could not observe any significant differences between the two groups in

Table 3. Incidence of recurrences in cases treated with intravesical CP and untreated cases

'T'/cases	CP treated group	Untreated group
CIS	3/4 (25%)	—
Ta	3/32 (9.3%)	16/30 (53.3%)
Tl	5/16 (31.2%)	8/14 (57.1%)
Total	11/52 (21.1%)	24/44 (54.5%)

terms of the degree of differentiation. Recurrences were less frequent in cases intravesically treated with CP (three with CIS, three with Ta and four with Tl) as compared with the untreated group (16 cases with Ta and eight with Tl) (Table 3).

In our patients treated with CP we did not find any relationship between the number of primary tumors, recurrences and response to immunotherapy. We noted progression in six cases – three in the group treated with CP (5.7%) and three in the untreated group (6.8%). None of the 52 patients treated with CP developed local or general complications.

The cytologic survey of cases treated with CP, normal and reactive post-TUR urothelial cells were frequently noted. Malignant cells were identified in 13 cases. In 23 out of 52 cases lymphocytes were present in a large number of smears. Granulocytes and red blood cells were rarely observed.

The histological lesions of the bladder wall are related specific to lamina propria. Initial tumors reveal a chronic inflammatory infiltrate in six out of 52 cases (11.5%), having a diffuse pattern and containing lymphocytes, granulocytes, plasma cells and rare macrophages; none of the 44 untreated cases presented any chronic infiltrate. After intravesical CP the chronic infiltrate containing mainly lymphocytes was present in the lamina propria of 36 out of 52 cases (69.2%); the infiltrate had a diffuse pattern, a folicular pattern and a pseudomembraneous pattern (continuous under the basal membrane). Of the patients which recurred, none presented such an infiltrate; from cases without recurrences, five did not have chronic infiltrate. High densities of lymphocytes were observed in Ta transitional cell carcinomas. White blood cells were occasionally noted in the thickness of the urothelium between epithelial cells.

Discussion

Over the last 20 years intravesical immunotherapy has demonstrated its efficiency in preventing

recurrences of superficial tumors of the urinary bladder. Many authors used BCG to reduce the rate of recurrences and even as a primary treatment of CIS.[12,13] Good results were reported in 38–65% of patients. The mechanism of action of BCG in human and animals and complications caused by this immunostimulant have been investigated.[14,15]

In order to increase the efficiency of immunotherapy and to reduce its complications other substances have been used, such as *Lactobacillus casei*, Levamisole and OK 432.[16] For this reason we administered intravesical CP, used as a potent immunostimulant, by a general route in some neoplasms as an adjuvant therapy. Results obtained in 52 patients treated in this manner showed a marked decrease of recurrences compared with the control untreated group of 44 cases; we identified recurrences in 21.1% in the untreated group and in 54.5% in the untreated group at follow-up after 3 years. The treatment with CP does not seem to influence progression, despite the reduced number of recurrences demonstrated. Results with CP are not related to the degree of differentiation, as was also observed for BCG.[17]

CP bacteria was isolated in 1926 by Mayer, but its action as an immunostimulant is not yet completely understood. Experimental studies showed a marked hyperplasia of the lymphomonocytic system after systemic administration.[18] The same mechanism could be involved in prevention of recurrences at the urinary bladder wall level, where it can induce lesions.[19] CP is effective in mice only if it is administered 10 days before inoculation of the mice with malignant cells; this protective effect may explain the failure of immunotherapy to reduce recurrences in cases with positive cytology after TUR. Urine cytology does not supply much information about the quality of responses in cases treated with CP, although we showed the presence of many lymphocytes in 23 patients. In cases treated with BCG, an increase of T helper cells and the T helper:T supressor cell ratio was described on bladder washings and in the bladder wall in patients with good response.[20,21] The most informative criteria to evaluate the efficiency of intravesical immunotherapy seems to be the histologic specimen. In view of the chronic infiltrate in the lamina propria we observed significant differences between treated and untreated patients. Few recurrences were noted in cases with such a histologic feature; folicular and pseudomembraneous patterns of the chronic infiltrate are particularly correlated with the absence of recurrences.

Conclusions

Periodic intravesical administration of CP vaccine induces morphological changes of the bladder wall in 69.2% of cases. Immunostimulant treatment with CP significantly decreases the rate of recurrences in superficial tumors of the urinary bladder. CP vaccine seems to be less effective on recurrences in cases with post-operative positive cytology for malignant cells. In cases with recurrences there were no significant differences between the incidence of progression in CP treated and untreated patients.

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