Anti-Cancer Drugs 1994, **5**, pp. 298–304

Combined immunochemotherapy (CEP, M-VEP + BCG) in the treatment of invasive bladder tumors

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Forty patients with invasive bladder tumors were consecutively treated and followed between June 1986 and February 1993. The treatment included systemic chemotherapy combining cyclophosphamide, epirubicin and cisplatin (CEP) or methotrexate, vinblastine, epirubicin and cisplatin (M-VEP) along with intravesically applied BCG vaccine. The treatment was well tolerated by the patients. No relevant toxic effects requiring hospitalization or fatalities due to the treatment were observed. Toxic manifestations of a hematologic nature were considerably less frequent than usual, nausea and vomiting being among the most frequently observed toxic signs on the second day of application of cisplatin. The side effects resulting from intravesically applied BCG vaccine showed no significant difference in terms of severity and variety from those due to its application in superficial tumors. A median follow-up of 50.3 months (range 6–80 months) showed an objective response to the treatment as follows: complete and partial response in 27 out of 40 (67.5%) and a complete clinical response in eight out of 40 (20%). Ten patients with partial response and stabilization had complete surgical response after operative treatment. The recurrence rate in patients with a complete response and a complete surgical response was 33% (six out of 18). The survival rate was 78% at 1 year, 70% at 2 years and 68% at 4 years. A complete response to the treatment of concomitant carcinoma *in situ* was observed in three patients. The lack of comparative and randomized studies and insufficient clinical experience did not allow an overall assessment of the therapeutic opportunities that our combined immunochemotherapy offers. The anti-tumor activity, moderate recurrence rate and comparatively good tolerance to this treatment indicate possibilities for multivariate immunochemotherapy in the treatment of invasive bladder tumors.

Key words: Bladder cancer, chemotherapy, immunochemotherapy, immunotherapy.

Introduction

Despite all achievements in the conventional treatment of invasive bladder tumors, i.e. surgical intervention and radiotherapy, the results as a whole are unsatisfactory, since a 5 year survival period does not exceed 20–40%. Distant metastases, which are the major cause of death in most cases, remain an unsolved problem. Radical cystectomy (with or without radiotherapy), yields unsatisfactory results, immediate operative risk and functional discomfort after application of various urine derivations. Some success has been achieved by the application of systemic chemotherapy. Combining cisplatin with other cytostatics has proven to be effective since an objective response to the treatment has been observed in 20–80% of the cases. The most effective combinations have proven to be methotrexate, vinblastine, adriamycin and cisplatin (M-VAC).[14] A noteworthy advance has been made in the immunotherapy of bladder tumors. Beside the well-proven effectiveness of intravesically applied BCG vaccine in superficial bladder tumors, there are experimental and clinical effects of new immunomodulators (interferon, keyhole limpet hemacyanin, OK-432, etc.) in combination with other therapeutic techniques.[4]

The idea of the combined application of cytostatics and immunomodulators dates back to 1969, when Mathe and co-workers reported successful application in children with lymphoblastic leukemia.[5] In 1986 we initiated a clinical study on the opportunities that immunochemotherapy offers in the treatment of invasive bladder tumors.[6] The relatively good results obtained with the first 10 patients who refused radical operative treatment or showed contraindications for it encouraged us to conduct further studies to determine the capacities of immunochemotherapy in invasive bladder tumors.

Materials and methods

From June 1986 through February 1993, 40 patients with advanced bladder tumors were treated and

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consecutively followed. They were given preliminary information concerning the nature and conditions of the treatment. In the initial period, the combination of cyclophosphamide, epirubicin and cisplatin (CEP) along with intravesically applied BCG was mainly used; a group of 30 patients was thus formed. Our clinical experience allowed us to form later a new group of 10 patients treated by systemic chemotherapy including methotrexate, vinblastine, epirubicin and cisplatin (M-VEP) plus intravesical BCG.

The clinical characteristics of the treated patients are given in Table 1. Pre-treatment clinical evaluation included bimanual examination, intravenous urography, chest X-ray, radionuclide scanning, sonography, computerized tomography (CT), cytoscopy with biopsy, urinary cytology, PPD, blood count (hemoglobin, leukocytes, thrombocytes), blood urea nitrogen, serum creatinine, alkaline phosphatase, SGOT, SGPT, liver function tests, ECG and sonocardiography. Control evaluation including sonography, CT, cytoscopy with biopsy and urinary cytology was carried out after the first month since initiating the treatment, and then every 3 months. Toxicity tests were performed before and after each course of treatment.

Treatment

Two therapeutic programs were applied: CEP—cyclophosphamide (600 mg/m^2; cyclophosphamide, Orion Corporation Farmos, Turku, Finland, Cyclophosphamide, Vebjenapharm Ankewerk Rudolstadt, Germany), epirubicin (30–50 mg/m^2; Farmorubicin, Farmitalia, Carloerba, Italy) and cisplatin (50–70 mg/m^2; Platidiam, Lechema, Brno Czechoslovakia) and M-VEP—methotrexate (30 mg/m^2; Methotrexat, Lechema, Brno, Czechoslovakia), vinblastine (2 mg/m^2; Vinblastine, Richter, Budapest, Hungary), epirubicin (30–50 mg/m^2) and cisplatin (5070 mg/m^2) (Figure 1). The dosage was reduced for patients with decreased numbers of leukocytes ($< 3000 \times 10$), thrombocytes ($< 1\,000\,009 \times 10$) and creatinine level > 1.5 mg%.

The treatment with the above combinations was repeated at intervals of 20–25 days in six consecutive cycles. In the cases when no response was observed up to the third cycle, the treatment was discontinued; in the event of a complete response, two additional cycles of multidrug chemotherapy were carried out. Three weeks after initiating the treatment, BCG (Calgevax; Research Institute of Infectious and Parasitic Disease, Sofia, Bulgaria) was applied intravesically in doses of four to six ampoules (0.6–1.8 $\times$ 10 microbial bodies). The vaccine was dissolved in 60 ml of physiological saline solution and installed into the bladder where it remained for 2 h. Six intravesical applications were performed at a rate of one application weekly, then reduced to one application monthly for a 1 year period (Figure 1).

Surgical treatment or radiotherapy were undertaken in patients with a partial response and stabilization of the disease, provided they consented to it. The type of operative intervention was determined taking into account the results of the treatment received, the principles of radicality and the patient's references. Incidences of recurrence after the therapy were treated surgically.

Table 1. Patient characteristics ($N = 40$)

Clinical parameters	CEP, M-VEP + BCG	
	N	%
Male/female ratio	37/3	
Age		
median	58	
range	40–73	
Performance status		
100–70	25	62.5
< 60	15	37.5
Stage		
T2	2	5
T3	22	55
T4	16	40
Grading		
1	—	—
2	12	30
3	28	70
Prior therapy		
Surgery	27	67.5
Radiation	—	—
Chemotherapy	3	7.5

Response criteria

A complete response was reported in cases of total disappearance of all measurable lesions having been proven physically, biochemically, radiologically and cytoscopically (by biopsy). A 50% reduction of the overall tumor mass is assumed to be a partial response; a less than 50% decrease of the tumor in size and a lack of new lesions are considered as stabilization of the disease. Progression of the disease leads to a 25% increase of a separate lesion in size or to the appearance of new lesions. The cases with complete disappearance of all known disease after operative treatment were

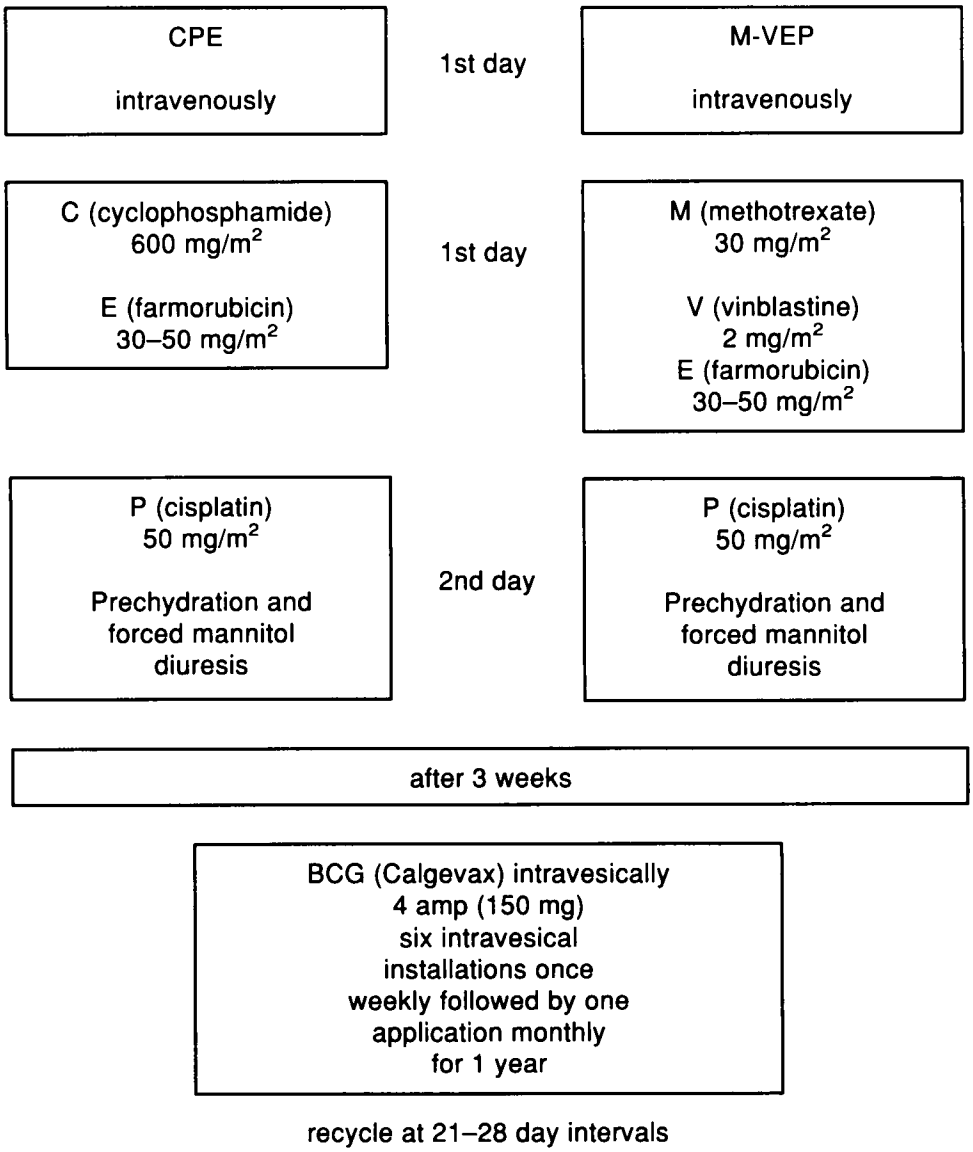

Figure 1. Scheme of CEP/M-VEP treatment in combination with intravesical BCG.

reported as surgical responses. Toxicity is graded according to WHO recommendations as follows: mild (grade 1), moderate (grade 2), severe (grade 3) and very severe (grade 4).[7].

Results

Among the 40 patients treated and followed, three refused further treatment after the third chemotherapy cycle. A total of 265 chemotherapy cycles were carried out for all patients: eight patients received three cycles each, and the remaining 32, four to eight cycles. The median follow up was 50.3 months (range: 6–80 months). All patients were subjected to toxicity tests (see Table 3). In none of the patients was the treatment discontinued on account of the side effects. The reason for discontinuing the treatment in three of the patients was rather of a psychological nature than related to toxicity. The most frequent complaints (in 95% of the cases) were nausea and vomiting, especially on the second day of the treatment in the course of cisplatin application. No cumulative immunosuppression was observed. Slight transient changes (2+) in the number of leukocytes in the course of the treatment were observed in two patients only. The median leukocyte count during the treatment was 7500 cells/mm for the whole group of patients.

No signs of thrombocytopenia were detected in any of the patients. The median serum creatinine level for the whole group was 119 mg% (range: 70–128 mg%) before the treatment and 124 mg% (range: 84–135 mg%) during the treatment. In six cases nephrotoxicity was observed during the treatment. Five of them exhibited slight serum creatinine elevations (range: 2.0 mg%). In three of the five the disturbances were transient, the other two had 2+ toxicity (serum creatinine level ranging between 2.0 and 3.5 mg%) which required discontinuation of the treatment after the third cycle. Reduced cisplatin doses were applied in patients with transient changes in serum creatinine level. Transient hematuria presenting no therapeutic problems was observed in eight of the patients after the second cycle. In most patients with profuse hematuria before the treatment accompanying the tumor process, chemotherapy was discontinued after the second cycle.

The common effects of intravesical application were observed, i.e. dysuria, elevation of the body temperature, feverishness, generalized fatigue, nausea, cystitis and hematuria. Their usual duration was 1–2 days. They showed no particular difference from those observed in BCG application in superficial tumors. However, a slight enhancement of the symptoms was noted after the sixth BCG installation as compared with BCG application in superficial tumors. Intravesical BCG application was discontinued in four patients at their request and in two patients on account of marked side effects. No hospital treatment of the side effects was applied for any of the patients. Complete and partial responses were obtained in 27 out of 40 patients (67.5%), stabilization in seven out of 40 (17.5%) and progression was observed in six out of 40 (15%).

The treatment results evaluated on the basis of some clinical parameters are given in Table 2. Ten patients with a partial response and stabilization had complete surgical response after operative treatment. Of eight patients with complete clinical response, only one had recurrent disease 49 months after completion of the treatment. Progression of the disease occurred later. The remaining seven patients are alive and were tumor-free for a period ranging between 6 and 60 months (median 34 months).

Progression of the disease was observed in four out of seven (57%) of the patients with a tumor mass having invaded more than two-thirds of the bladder wall. Overall, in these patients, the survival duration was not more than 2 years.

The duration of remission in patients with complete clinical response and complete surgical response had a median of 31 months (range: 373 months). In five out of 10 patients with complete surgical response (50%), recurrent tumors of medium size were observed and successfully treated again by surgical intervention. Ten patients died of the main disease and three of other causes. Four patients were not followed afte the second year since operation.

Complete response was observed in two patients with concomitant carcinoma *in situ* and partial response in one of them.

The overall survival rate among the patients treated with immunochemotherapy was 78% at 1 year, 70% at 2 years and 68% at 4 years.

Discussion

After the demonstration that the combination of cytoreductive chemotherapy with BCG vaccine leads to long term remission and survival in children with lymphoblastic leukemia,[5,8] a number of experimental works on various tumor models have confirmed the therapeutic potential of immunochemotherapy.[9,13]

The main goal of immunochemotherapy is to reduce the tumor mass and to stimulate the lymphoreticular system. In 1979, Adolphs *et al.*[14] reported a synergic effect produced by consecutive applications of cyclophosphamide and BCG on chemically induced tumors in rats. From this they concluded that cyclophosphamide application causes a suppression of the humoral immunity response which results in a decrease in immune complex formation. On the other hand, the cell-mediated immune response is stimulated by way of inhibiting the suppressor cell precursors. Furthermore, the tubercular bacteria produce non-specific stimulation of cell immunity.

An immunohistologic study on local immunity after intravesical application of BCG has been conducted by Boccafoschi *et al.*[15] It established a prevalence of T lymphocytes on account of altered proportions between helpers and suppressors (CD4/CD8 > 1). However, the absence of any difference in lymphocyte phenotype between responders and non-responders to BCG indicates that the local mechanism is not the only factor responsible for the anti-tumor effect of the vaccine.[15]

Studies on the effect of BCG on invasive bladder tumors[16] proved that *in vitro* BCG application on the invasive EJ cell line in humans inhibits the invasion of these tumor cells. It was established that the vaccine influences the tumor cell mobility.[16]

Table 2. Response to the treatment regarding patient charateristics

Clinical parameters	Total no. of patients	Response rates									
		CR		PR		SD		PD		CRs	
		N	%	*N*	%	*N*	%	*N*	%	*N*	%
T2	2	1	50	1	50	—	—	—	—	—	—
T3	22	4	18	13	59	4	18	1	5	9	41
T4	16	3	19	5	31	3	19	5	31	1	6
G2	12	4	33	7	58	1	8	—	—	3	25
G3	28	4	14	12	42	6	22	6	22	7	25
Paravesic invasion	20	5	25	10	50	2	10	3	15	—	—
Prostatic invasion	3	—	—	2	67	—	—	1	33	—	—
Prior surgery	27	3	11	13	48	6	22	5	19	6	22
No prior surgery	13	5	38	6	46	1	8	1	8	4	31

Wooley *et al.*[17] established that tumor recurrence was considerably reduced by combined application of *Corinebacterium parvum* and cisplatin in mice transitional cell tumors (MB-T). Simultaneously, a considerable increase in phagocytic natural killer activity primarily in the inguinal lymph nodes was observed. The authors noted that the mode of *C. parvum* applications (local or systemic) is important to control the local tumor process.

Dekernion *et al.*[18] combined citoxan and BCG in experimental FANFT-induced tumors in mice. Unlike Adolphs *et al.*,[14] they did not establish potentiation of the anti-tumor effect of citoxan by BCG, but observed inhibition of tumor growth and an increased survival rate in animals treated with citoxan and *C. parvum.*[18] These contradictory results may be attributed to differences in the experimental models. Studies conducted by Currie and Bagshawe[19] demonstrated the significance of a number of factors: sequence, time of treatment and treatment duration. The authors assumed hat the introduction of *C. parvum* 12 days after initiating cyclophosphamide treatment was successful in 70% of the animals with fibrosarcomas, whereas other sequences and changes in the therapeutic program proved to be unsuccessful.[19] Beyond any doubt, a thorough study on the role of immunochemotherapy considering a number of factors (e.g. tumor biology, selection of treatment components according to their mechanism of action, dosage and duration of application) is essential for assessment of the actual potentialities of immunochemotherapy.

Table 3. Toxic signs of immunochemotherapy (CEP, M-VEP + BCG)

	N	%
Toxic signs of chemotherapy		
cardiotoxicity	2	5
nausea and vomiting	38	95
alopecia	2	5
diarrhea	8	20
dysuria/hematuria	8	20
fatigue	22	55
leucocytopenia	2	5
nephrotoxicity	6	15
Toxic signs of intravesical BCG		
dysuria	40	100
hematuria	28	70
fever	23	58
malaise	48	45
cystitis	2	5
fatigue	9	22
epididimytis	2	5

After obtaining encouraging results with the first 10 patients with advanced bladder tumors refusing surgical treatment or with contraindications for it, we did further studies including patients beyond the initial conditions of this group. Our immunochemotherapy programs applied to advanced bladder tumors proved to be well-tolerated by most of the patients. No relevant toxic manifestations or mortality resulting from the treatment were observed.

The treatment was discontinued after the third chemotherapy course in one of the patients only, on account of persistent nephrotoxicity. The most frequent complaints were observed on the second day of cisplatin application, but they can be reduced by adequate medication. Lately, we have made successful use of ondansteron applied intravenously

immediately before application of cisplatin. The results from our toxicity tests were not significantly different from those reported in the literature concerning the separate application of the two components of the treatment.[2,3,6,14,18]

The side effects of intravesical BCG showed no significant difference in severity and variety from those due to the application of BCG in superficial tumors. The side effects were more intensively manifested after the first six installations applied as supporting therapy, which required discontinuation of the treatment in two patients. The reason for discontinuing the treatment after the third course in three other patients was not related to toxic effects. No significant difference in terms of toxicity was observed as a result of the application of the two chemotherapeutic combinations (CEP and M-VEP).

A complete objective response to both immunochemotherapeutic variants applied was registered in 24 out of 40 (67.5%) of the cases. Since only a small number of patients have been treated with M-VEP and intravesical BCG so far, no practical evaluation of the results obtained by applying the two therapeutic programs is possible Our preliminary impression is that these results present no significant difference.

The relationship between the results obtained in some clinical parameters is shown in Table 2. Except for the few patients with stage T2 tumors, the percentage of objective responders with stage T3 was 77% against 50% for those with stage T4. Still greater differences were noted when comparing the grading to the treatment results: 11 objective responders out of 12 G2 patients (91%) against 11 objective responders out of 28 G3 patients (57%). The fact that among the patients having received prior operative treatment (TUR and partial rejection) 16 out of 27 (59%) showed no objective response against 11 out of 13 (85%) of those who had not received such treatment confirmed our observation that prior operative treatment reduces the efficacy of subsequent immunochemotherapy. The overall tumor size is also an essential factor in the issue of the treatment: progression of the disease was observed in four out of seven (57%) of the patients with a tumor mass having invaded more than two-thirds of the bladder wall. A survival duration of more than 2 years was observed in none of them. In the patients with complete surgical response, those with stage T3 prevailed—nine out of 10 (90%). The median remission duration in patients with complete clinical response and complete surgical response was 31 months (range: 3–73 months). The lowest recurrence rate after chemotherapy was noted in patients with complete clinical responseone out of eight (12%), while in those with complete surgical response it was five out of 10 (50%). After recurrence, progression of the disease was observed in one complete responder and in one patient with complete surgical response.

The treatment results obtained in three patients with concomitant carcinoma *in situ* are of some interest: after immunochemotherapy, a complete response was observed in two of them and a partial response in one. Subsequent operative treatment of the latter showed no presence of carcinoma *in situ*.

The survival rate among the patients was 78% at 1 year, 70% at 2 years and 68% at 4 years. Recently, the study was extended by including a greater number of patients with stage T3 compared with those in stage T4, which reduced considerably the number of fatalities and reflected upon the total survival rate of the patients treated. For the time being, we assume that in stage T2T3 tumors invading not more than two-thirds of the bladder wall and concomitant carcinoma *in situ*, a lack of prior operative treatment and unfavourable localization for a bladder preserving operationimmunochemotherapy is a good therapeutic alternative.

The relatively low recurrence rate in patients with complete clinical response and complete surgical response (33%), as well as the possibility of obtaning a good therapeutic effect on tumors with unfavorable localization for a bladder preserving operation, are also noteworthy. A study on the potentialities of immunochemotherapy and organ-preserving operations in bladder tumors is being conducted.

As a whole, the results we obtained by applying immunochemotherapy in advanced bladder tumors show the lowest percentage of complete and partial responders compared with the results of different applications of analogic cytostatic combinationsCIS-CA and M-VAC.[3,20,21] In our opinion, these differences are due to the non-identical clinical characteristics of the patients treated and to differences in the therapeutic programs (dosage, drugs used, etc.), as well as to different response criteria. An overall assessment of the potentialities of immunochemotherapy requires randomized comparative studies on the application of immunochemotherapy and separately applied chemotherapy.

Our clinical experience in the application of immunochemotherapy in advanced bladder tumors, despite the good results obtained, does not allow us to predict with confidence the therapeutic potential of immunochemotherapy. The anti-tumor

activity observed, relatively low recurrence rate and comparatively good tolerance to treatment suggest a possible use of randomized studies in future.

Acknowledgments

We thank Mr T Bell of the British Council, Sana'a, Yemen, for assistance in the preparation of this manuscript.

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(Received 16 August 1993; received in revised form 24 January 1994; accepted 21 February 1994)