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A pilot study comparing different dose levels and administration schedules of interferon-α2b combined with epirubicin for prevention of recurrence in bladder cancer

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A pilot study in 62 patients has been carried out to evaluate the combination of epirubicin and interferon (IFN)-α2b using different doses and schedules of intravesical administration in the prevention of recurrence of bladder cancer. Preliminary results show greater prophylactic efficacy not only when higher doses of epirubicin and IFN-α2b are used but also when a larger interval between instillation of the compounds was used.

Key words: Bladder cancer, combination therapy, epirubicin, interferon-α2b

Introduction

No significant progress has been achieved in recent years in the field of the topical treatment of superficial bladder tumors. Despite the sagacious use of the currently available chemotherapeutic agents, the recurrence rate after transurethral resection (TUR) has been reduced but not fully abolished and progression to higher stages still occurs.[1,2] Due to a better understanding of the complex mechanisms of interaction between the tumoral cell and the patient's immune system, several agents able to increase the immune response have been introduced. In spite of the number of agents tested intravesically, only *Bacillus Calmette-Guérin* (BCG) therapy has so far been proved to be consistently effective in the intravesical therapy and prophylaxis of superficial bladder tumors.[3–5] The immunologic mechanism by which BCG acts is complex and not completely understood, but recent work shows that an increase in lymphokine urinary concentration can be observed after BCG intravesical administration.[6] Thus, BCG may represent a nonspecific stimulus producing an immune response mediated by different factors, such as interferons (IFNs) and interleukins.

The intravesical use of biologic response modifiers, such as IFN-β,[7] interleukin-2,[8] and IFN-α[9–12] is in a preliminary phase of study. Ikic *et al.*[9] obtained two complete and two partial responses in eight patients with superficial transitional cell carcinoma (TCC) of the bladder using intratumoral injection of IFN-α, at a dose of 2 MU daily for 21 days. Shortliffe *et al.*,[10] using weekly intravesical instillation of IFN-α at a dose varying from 50 to 200 MU per instillation, achieved a complete response in four of eight patients with T_{is} and in one patient with both T_a papillary TCC and T_{is}. The same authors could achieve no complete responses in four patients with papillary T_a-T_1 TCC. Torti *et al.*,[11] in 1988, adopted a dose escalation approach using IFN-α in doses from 50 to 1000 MU and reported a 31% complete remission rate in 35 patients with G1–G2 T_a-T_1 papillary TCC or T_{is}. In the same year, Ackermann *et al.*,[12] with weekly instillation of IFN-α2a at a dose of 54 MU reported two partial responses in five patients with T_a TCC and one complete and one partial response in four patients with T_{is}. In a randomized, controlled trial in 115 patients with T_{is}, Chodak[13] reported a significantly higher rate of complete response to high-dose (100 MU) than to low-dose (10 MU) IFN-α2b (45% versus 6%, respectively). IFN was administered intravesically every week for 12 weeks and then monthly for 1 year.

The rationale for the sequential intravesical treatment with epirubicin and IFN-α2b

The combination of different anticancer drugs in the systemic chemotherapy of advanced bladder

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cancer has been demonstrated to be more effective than the use of a single agent. In the same way, it is hoped that better results can be obtained by use of a topical combination of various agents with synergistic action and lacking cross-resistance.[14] Doxorubicin and epirubicin have been proved to be effective against superficial bladder cancer when used intravesically[2,15–17] and are well established in clinical use. The intravesical use of IFN-α in the treatment of papillary superficial bladder tumors has not yet shown superiority over conventional agents.[18] Moreover, its action against carcinoma *in situ* seems to be strictly dose-related.[13] However, it is possible that the combination of IFN with other drugs allows a reduction of the effective dose and a better efficacy against papillary tumors as preliminary experience suggests the possibility of a synergistic action between chemotherapeutic agents and lymphokines. In experimental animals synergism seems to occur with tumor necrosis factor α and mitomycin C,[19] between interleukin-2 and decarbazine,[20] and between IFN-α and doxorubicin.[21–23]

An immunomodulation with IFN is possible only if an adequate lymphocyte population is present at the tumor site. The role of an anticancer drug used in combination (e.g. epirubicin) would be to promote tumor necrosis, inflammation and tumor infiltration by lymphocytes. The sequential use of IFN-α2b may then realize a lymphokine activated tumor inhibition (LATI) system. LATI is a non-tumor-specific phenomenon observed in transplantable murine tumors; in mice challenged with tumor cells admixed with lymphocytes from tumor-bearing mice, a complete tumor inhibition takes place when lymphokines are injected around the tumor area. This effect does not occur if lymphocytes are not admixed with tumoral cells. The chain of events leading to a LATI system starts when an exogenous lymphokine binds with specific receptors of the lymphocytes admixed to tumoral cells. In the presence of exogenous lymphokines, the lymphocytes may acquire lymphokine-activated killer cell activity and kill tumor cells. They also deliver various lymphokines and recruit several host lymphocyte populations. Thus, LATI system, even if a non-specific reaction, may result in a tumor-specific delayed type of hypersensitivity and immune memory.[24,25]

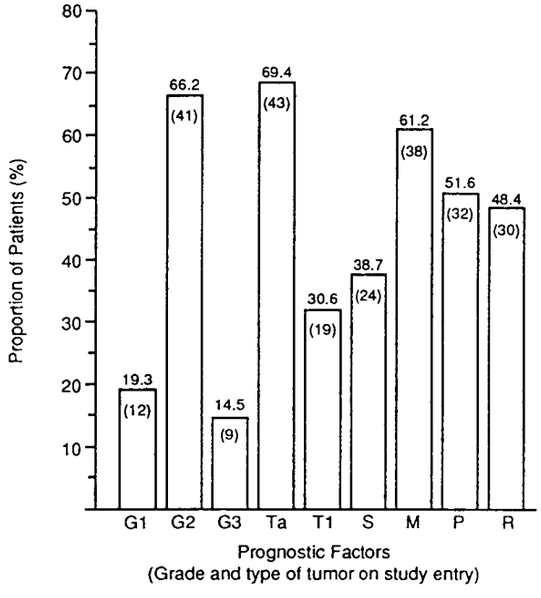

Figure 1. Patient characteristics on study entry. Tumors are graded (G1–G3, T_a and T_1) and classified according to type: S = single, M = multiple, P = primary, R = recurrent. The number of patients in each category is expressed both as a numeric total (in parentheses) and as a percentage of the 62 patients in the study.

Personal experience

Materials and methods

Preliminary experience with the intravesical combination of IFN and epirubicin for bladder cancer has shown encouraging results.[22,23] At the Institute of Urology of Palermo, 62 patients have been introduced to a pilot study to compare different doses and schedules of a sequential combination of epirubicin and IFN-α2b in the intravesical prophylaxis of superficial bladder tumors after TUR.

Patients' characteristics at entry are shown in Figure 1. They were patients at high risk for recurrence since 50% of them had recurrent and pretreated tumors, more than 70% had multiple and G2 or G3 tumors. At least one of these unfavorable prognostic factors was present in each patient. Mean number of lesions was 1.7 (range 1–9) and mean recurrence rate at entry was 1.4 per year.

Patients were distributed into four groups, comparable for the main prognostic factors (Figure 2), according to different modalities of treatment:

Group 1: epirubicin 30 mg + IFN-α2b 5 MU after 1 h (15 patients)

Group 2: epirubicin 30 mg + IFN-α2b 5 MU after 24 h (16 patients)

Group 3: epirubicin 50 mg + IFN-α2b 10 MU after 1 h (18 patients)

Group 4: epirubicin 50 mg + IFN-α2b 10 MU after 24 h (13 patients)

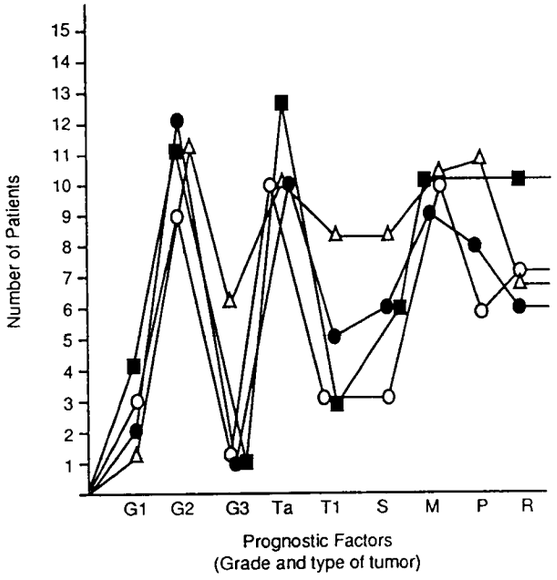

Figure 2. Distribution of the main prognostic factors in the four groups. Tumors are graded (G1–G3, T_a and T_1) and classified according to type: S = single, M = multiple, P = primary, R = recurrent. Groups are designated as follows: Group 1, ■; Group 2, △; Group 3, ○; Group 4, ●.

Both drugs, diluted in normal saline solution, epirubicin at the concentration of 1 mg/ml and IFN-α2b in 30 ml, were maintained into the bladder for 1 h. Both drugs were given weekly for the first month, then for the following 5 months epirubicin was given monthly while IFN was administered every 15 days.

To determine whether a progressive increase occurred after the sequential intravesical administration of epirubicin and IFN-α2b, urinary concentration of interleukins (IL-1, IL-2, IL-4 and IL-6) was determined in 10 patients each from Groups 3 and 4. The schedule of urine storage is given in Table 1. A total of 40 assays was performed for each patient. All urine samples (30 ml) were centrifuged at 200 r.p.m. for 15 min, concentrated 10-fold using millipore filters and then stored at −70°C within 2 h after collection. IL-1, IL-2, IL-4 and IL-6 determination was performed by the ELISA procedure. Briefly, urine samples and standards were incubated on 90-well plates coated with interleukin-specific monoclonal antibody for 18 h at 25°C. After three washes, plates were incubated with anti-IL-1, -IL-2, -IL-4 or -IL-6 specific antisera for 18 h at 25°C and then with enzyme conjugated antibody for 4 h at 37°C. Substrate reaction was measured and related to the adsorbance of the standard curves.

Table 1. Schedule of urine storage for determination of interleukins

Urine storage at the following times:
(a) At entry
(b) At the first, fourth and eighth instillation
At the first, fourth and eighth instillation urine is taken as follows:
(a) Before the instillation
(b) After 2 h from EPI instillation
(c) After 2 h from IFN-α instillation
(d) After 4 h from IFN-α instillation

Results

At a mean follow up of 24 months (16–30 months), 37 patients (59.6%) showed a recurrence. Mean recurrence rate was 0.48 per year (0.55 in patients with previous recurrences). The mean number of tumors was three (range 1–9). The percentage of recurrences is distributed in the four groups as follows: Group 1, 86%; Group 2, 56%; Group 3, 55%; Group 4, 43% (Figure 3). The percentage of recurrences, at mean follow up of 9 and 24 months in each group and according to dose and interval between the instillation of epirubicin and IFN-α2b, is given in Table 2. No significant

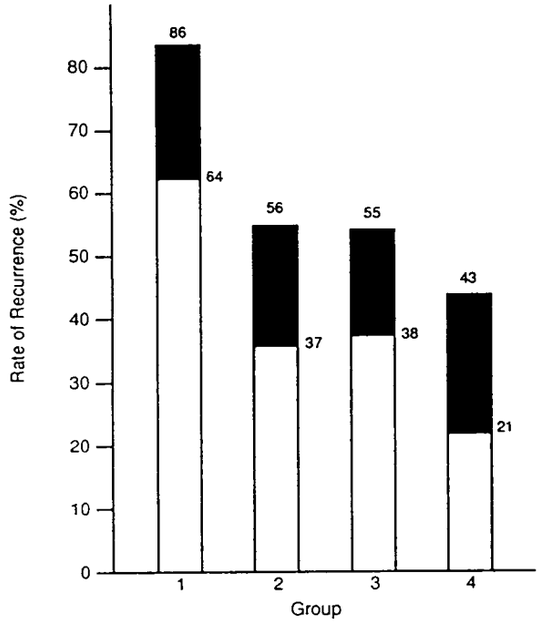

Figure 3. Percentage recurrence in relation to treatment. Follow-up periods were 9 (□) and 24 (■) months.

Table 2. Percentage of recurrence according to dose and schedule

Mean follow up	9 months	24 months
Group 1	64	86
Group 2	37	56
Group 3	38	55
Group 4	21	43
Low dose	46	70
High dose	28	50
Short interval	47	68
Long interval	25	50

difference was evident in percentage of recurrences according to the main prognostic factors (Figure 4). Tolerability was excellent since in no case was it necessary to stop the treatment. Mild-to-moderate chemical cystitis was present in 12 patients (19.3%). The preliminary results of urinary interleukins determination (the research is still ongoing) reveal a moderate increase of IL-2 and IL-4 concentrations in patients who received IFN-α2b 24 h after epirubicin instillation (Group 4). This trend is not present in patients who received IFN-α2b 1 h after epirubicin (Group 3).

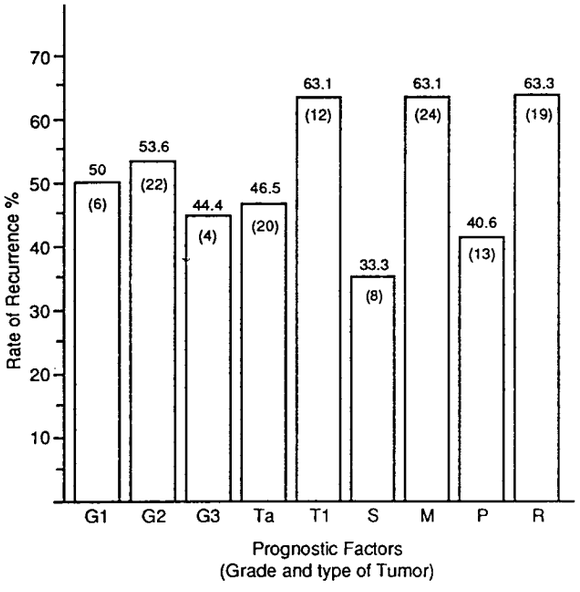

Figure 4. Percentage of recurrence according to the main prognostic factors. Tumors are graded (G1–G3, T_a and T_1) and classified according to type: S = single, M = multiple, P = primary, R = recurrent. The number of patients in each category is expressed both as a numeric total (in parentheses) and as a percentage of the 62 patients included in the study.

Conclusion

The results obtained in this pilot study seem to show the efficacy of the intravesical sequential use of epirubicin and IFN-α2b. The groups were not randomized and the numbers were small but there was an even distribution of the main prognostic factors. The recurrence rate decreased from 1.4 to 0.5 per year in patients with recurrent tumors. The preliminary results seem to show a greater prophylactic efficacy not only when the higher doses of both epirubicin and IFN-α2b were employed, but also when a longer interval between the instillation of epirubicin and interferon was adopted. Our laboratory research seems to confirm these results since an increase in urinary concentration of interleukins is detected only in patients who received IFN 24 h after epirubicin instillation. These data need to be confirmed by more extensive research since in our study determination of urinary interleukin levels was performed not longer than 4 h after IFN instillation and higher levels are probable after a longer interval. Another issue emerging from this part of the study is the trend to a flattening of the differences obtained with different doses and schedules as the interval of time from the end of the intravesical prophylaxis is prolonged. This event could be explained by the progressive decrease of activated lymphocytes in the absence of immunomodulation.

It is suggested therefore that such an approach should be employed in further comparative studies to assess the efficacy of the combination versus that of epirubicin alone. Such a comparative randomized trial has been designed and will be activated soon in Italy.

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