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INTRAARTERIAL CHEMOIMMUNOTHERAPY IN REGIONALLY ADVANCED PELVIC MALIGNANCY

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Abstract

A prospective clinical trial combining locoregional chemoimmunotherapy for locally advanced pelvic malignancies in 28 patients was conducted, all patients were subjected to exploration for insertion of an arterial catheter and the other end of the catheter was connected to a subcutaneously implanted valve in the lower abdomen. After twelve days a bolus injection of intraarterial chemotherapy using cisplatin (50 mg/m2), Mitomycin (6 mg/m2) for bladder carcinoma. Doxorubicin (50 mg/m2) and cisplatin (50 mg/m2) for ovarian carcinoma and cisplatin (50 mg/m2) and fluouracil (50 mg/m2) for colorectal carcinoma were given for one day, then on the fifteen days the patient receives autologous lymphokin activated killen cells by intracatheteric injection over 15 minutes. In the 22nd day after the exploration 1 ml of interleukin-2 was injected via the catheter for 3 days . One week after the end of this regimen, the same course can be repeated for another course if there is response . The overall response was 82.1% (23 patients, CR 3.6 % & PR 78.5%) the remaining 5 patients had stable disease (17.9%), the difference was statistically significant P <0.01. The responding patients (23 cases) were subjected for surgical management . Complications secondary to the treatment regimen used were accpetable and did not-lead to any morbid consequences. So, the locoregional chemoimmunotherapy regimen employed was well tolerated with a good overall response rate and without any significant clinical morbidity .

Introduction

Regionally advanced pelvic malignancies are unresectable, thus radiotherapy and chemotherapy are the fundamental treatment methods, however these regimens have failed to diffuse to the tumour properly . Also lowered immunity in cancer patients add to the difficulty in management of these cases. It is likely that immunorestoration alters considerably the outcome of antineoplastic therapy since immunocompetent patients have a better prognosis (Fujiwaki et al, 1997). Locoregional chemoimmunotherapy possesses the ability to effectively concentrate the drugs in the diseased segment . Moreover, it spares the remaining non diseased tissues by being exclusively retained in the space occupied by the tumour (Lygidakis et al, 1998). Combined chemoimmunotherapy lead to : first, induction of anatomic locoregional vascular disruptions which inevitably lead to necrosis of tumour burden, second, locoregional transfer of both cellular and humoral elements, thus inducing regional blood supply alterations and augmentation of the patient s immunocomptence by instigating a cascade of immune responses directed against the tumour (Lygidakis et al, 1998). Several studies of intraarterial chemoimmunotherapy followed by surgery and or radiotherapy in locally advanced pelvic malignancy have demonstrated high rates of clinical and histologic responses (Edward et al, 1994 and Sueyama et al, 1995), and have improved responses rates over that of conventional chemotherapy (Lygidakis et al., 1995).

So, the aim of this work was to confirm the antitumour activity of regionally infused chemotherapy plus autologous lymphokine-activated killer cells as a test for new cancer treatment & improves the tolerance to cytotoxic combination therapy by using LAK cell for helper cell activation.

Materials and Methods

Patient Criteria :

This study was done from January 1996 to January 1998, at Surgical Oncology and Clinical Oncology & Nuclear Medicine Departments, Mansoura University , 28 evaluable patients with locally advanced pelvic malignancy were

studied. Patient characteristics are shown in table (1). Informed consent from all patients to receive the proposed treatment was taken . Criteria for eligibility included histologically proven malignancy, unresectability, absence of distant metastasis, absence of associated uncompensated diseases (heart, respiratory, hepatic or renal) no concomitant corticosteroid therapy, or serious infections necessitating antibiotics. Staging procedures included clinical examination, pelvic CT or MRI, chest radiograph, intravenous pyelogram, cystoscopy, sigmoidoscopy, abdominal CT and tumours markers as CEA, CA19-9 and CA125.

Control group are 10 normal persons in whom OKT3 (Total T cell subsets), OKT4 (Helper T cell) and OKT8 rates (suppressor T cell) were investigated .

Treatment Plan :

1- Surgery :

Under general anaesthesia exploration was done. Careful staging of the disease following U. I. C. C. criteria is necessary .Moreover the presence of ascites or peritoneal nodules in cancer ovary should be recorded. The internal iliac., the superior rectal or the ovarian arteries should be identified, dissected, isolated and its proximal part should be ligated. the other part should be opened and an arterial catheter should be advanced towards the rectum, the ovary, or the bladder and secured with three silk ligatures in place. Patency of the catheter is assured by using heparinized water solution. The other end of the catheter is connected to a subcutaneously implanted valve in the lower abdomen.

II. Post-operative strategy :

* One week after surgery , 500 ml of patient s blood is taken & immediately replaced by the same amount . Lymphocytes are separated & cultured with Interleukin-2 (IL-2) for one week.

Preparation of LAK cells :

500 ml of blood are obtained, diluted with equal volume of RPMI 1640, centrifuged over ficoll-Hypaque. Mononuclear cells are harvested, washed and then calculated each 1 million peripheral blood lymphocytes were cultured in 1 ml media (RPMI 1640, foetal

calf serum 10% penicillinstreptomycin 2% & 100 unit IL-2) and cultured in humidified atmosphere of 5% CO^2 in air at 37°C for 1 week, then separation after centrifugation & counting by hemocytometer in the day of injection (Rosenburgh et al., (1986) .

LAK Administration :

Patients received the cells by intra-catheteric injection over 15 minutes after dilution in 50 ml of 5% dextrose with a total number of about 3x109 cells.

* Twelve days after surgery, a bolus injection of the following chemotherapy drugs is given.

* For carcinoma of the bladder : Cisplatin (50 mg/m^2) & Mitomycin (6 mg/m2)

* For colorectal carcinoma : Cisplatin (50 mg/m^2) & 5-fluouracil (500 mg/m^2).

* For ovarian carcinoma : Doxorubicin (50 mg/m^2) Cisplatin (50 mg/m^2).

* Fifteen days after the exploration, the patient receives autologous lymphokine activated killen cells which were cultivated in vitro from the patients in the study, the number of cells about $3x10^9$ LAK cells.

* In the 22nd. Day after the exploration, 1 ml. of proleukin 18x106 IU is injected via the catheter for 3 days .

* One week after the end of this regimen, the same treatment can be repeated if there is response.

Criteria for stopping the treatment :

respiratory distress, major anaphylaxis, severe proctitis, severe diarrhea, bleeding per rectum, massive haematuria or oliguria.

III- Assesment of response :

Response to treatment was monitored, complete response (CR) was defined as the reduction of > 75% of the tumour mass and normalization of tumour marker levels, a partial response (PR) as a reduction of > 50% of the tumour mass and tumour marker reduc-

tions as evidenced by abdominal CT or MR and blood serum determination, stable disease (SD) as an average decrease of less than 50% in the above mentioned parameters, while progressive disease (PD) as a further increase in tumour mass or the appearance of new lesions or further elevations of tumour marker.

After 2 courses, the responding patients are subjected for surgical management. Before surgery : routine investigations, CT pelvis & metastatic workup to exclude remote metastasis. Determination of T-cell subsets; OKT_3, OKT_4 & OKT_8. During surgery : strict localization and mapping of the tumour, absence of abdominal or pelvic metastasis other than pelvic lymph nodes. During chemoimmunotherapy : blood picture, liver and kidney functions every other day. After treatment : routine investigations, CT pelvis, metastatic workup, determination of T-cell subsets and tumour markers .

Statistical analysis :

All patients eneterd on the study were monitored for treatment related response by test of proportionates and student s t-test to compare group.

Results

The overall response rate was 82.1% (23/28), complete response in one patient (3.6%) and partial response in 22 patients (78.5%) , as shown in figure (3 &4) the remaining 5 patients had stable disease (17.9%), the difference was statistically significant ($P < 0.01$).

The total T-cell rate (OKT^3) of circulating lymphocytes in patients with locally advanced malignancy before treatment was found to be significantly decreased compared to normal individuals laboratory values which was found to be 70.14 ± 3.34 and 85.3 ± 3.43 respectively ($P < 0.01$, figure 1 and table, 3) stimulation caused by treatment had a significant effect on OKT_3 production causing an increase in levels from 70.14 ± 3.34 to 92.36 ± 3.54 after treatment ($P < 0.01$, figure 2 and table 4). The helper phenotype substitution OKT_4, markedly increased in most of the patients from 35.50 ± 2.41 to 65.18 ± 3.74 (table 4) after treatment which was statistically significant ($P < 0.01$) to reach the

normal level presented in the control group with mean value 66.80 ± 2.39 (table 3). The suppressor cell OKT8 rate was higher in cancer patients in relation to control group (29.14±1.74 and 24.30 ± 2.50 respectively) this relation was statistically significant ($P < 0.01$, table 3) and reduced again after treatment to 26.93 ± 1.84, These reduction was statistically significant ($P<0.01$, table 4) .The helper OKT_4 suppressor OKT8 ratio was markedly increased from 1.22 ± 0.11 to 2.43 ± 0.5 after treatment which was statistically significant ($P<0.01$, table 4) indicating improve immune status after LAK infusion .

Complications secondary to treatment regimen used were acceptable and consisting of Nausea & vomiting in 78.5% of cases, diarrhea and dysentery 53.5%, haematuria in one patient . Haematological complications were mild and included anaemia 7%, leucopenia 10.5% and thromhocytopenia 7% (table 5). Elevation of serum creatinin occurred but within normal limits (range 1.2-1.5 mg/dl). One patient suffered from mucositis. No catheter infection or dislodgment and tearing of the arterial wall. Surgery was done to 23 responding patients, radical surgery was feasible in 18 patients only (64%) , the other 5 patients showed pelvic wall infiltration. Histologic examination of the surgically resected specimens was done to assess the extend of disease and the status of surgical margins. Eleven out of 18 patients (61%) showed marked response as indicated by necrosis, cancer free surgical margins were obtained in 16 patients. For any tumour remained in the histologic specimen and no free surgical margin, external radiotherapy to the whole pelvis (total dose 40-50 Gy) and/or systemic chemotherapy was given postoperatively .

Table 1 : Patient Characteristics.

Patient characteristics	No. of patients n = 28	%
Age mean (ange)	55 (43-64)	
Sites		
Colorectal	12	42.8%
Bladder	8	28.6%
Ovaies	8	28.6%
Histologic Types		
Adenocacinoma	24	85.7%
Squamous cell carcinoma	4	14.3%
Grade of diffeentiation		
Well-diffeentiated	6	21.7%
Moderately differentiated	17	60.7%
Poorly differentiated	5	17.9%

Table 2 : Response rate to chemoimmunotherapy in 28 patients .

Type of response	No. of patients	%	P
Responders (overall response)	23	82.1	< 0.01
Stationary disease	5	17.9	
Total	28	100.0	

Table 3 : Comparison between Cancer patients before treatment and controls according to OKT_3, OKT_4, OKT_8 and OKT_4/OKT_8.

	Control	Before Treatment	t	P
	X ± SD	X ± SD		
OKT_3	85.30 ± 3.43	70.14 ± 3.34	12.879	< 0.01
OKT_4	66.80 ± 2.39	35.50 ± 2.41	35.296	< 0.01
OKT_8	24.30 ± 2.50	29.14 ± 1.74	6.726	< 0.01
OKT_4/OKT_8	2.77 ± 0.27	1.22 ± 0.11	25.256	< 0.01

Table 4 : Effect of chemoimmunotherapy on OKT3, OKT4, OKT8 and OKT4/OKT8 levels.

	Control	Before Treatment	t	P
	X ± SD	X ± SD		
OKT_3	70.14 ± 3.34	92.36 ± 3.54	12.877	< 0.01
OKT_4	35.50 ± 2.41	65.18 ± 3.74	35.290	< 0.01
OKT_8	29.14 ± 1.74	26.93 ± 1.84	20.624	< 0.01
OKT_4/OKT_8	1.22 ± 0.11	02.43 ± 0.15	44.628	< 0.01

Table 5 : Complications to treatment .

Complications	Number of patients (n = 28)	%
Nausea & Vomiting	22	78.5%
Diarrhae	15	53.5%
Haematuria	1	3.5%
Anaemia	2	7%
Leukopenia	3	10.5%
Thrombocytopenia	2	7%
Mucositis	1	3.5%

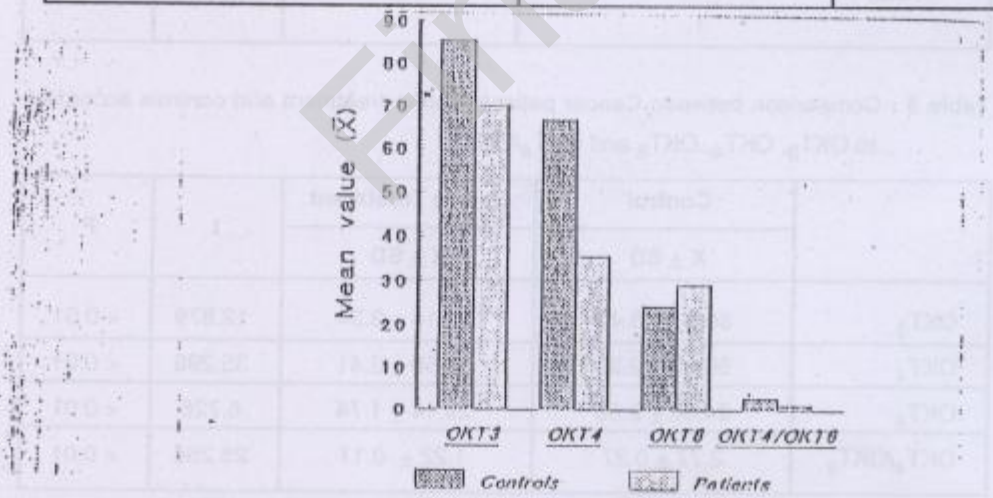

Fig. 1 : Comparison between cancer patients & Controls .

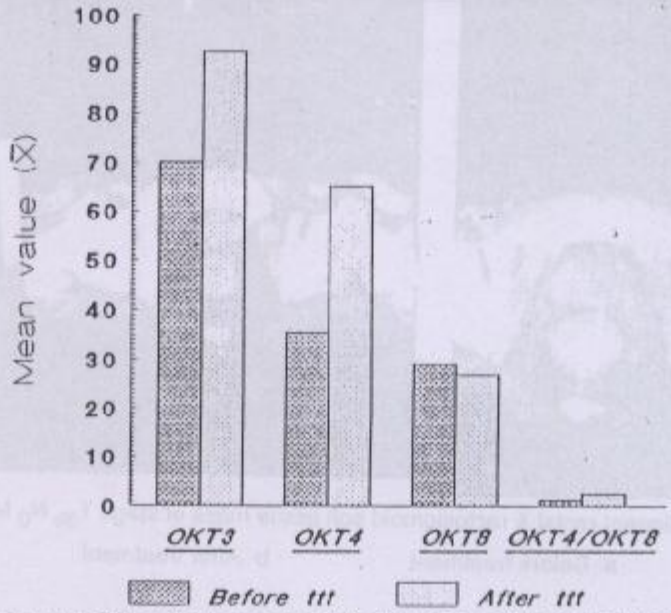

Fig. 2 : Effect of treatment of different parameters among cancer patients.

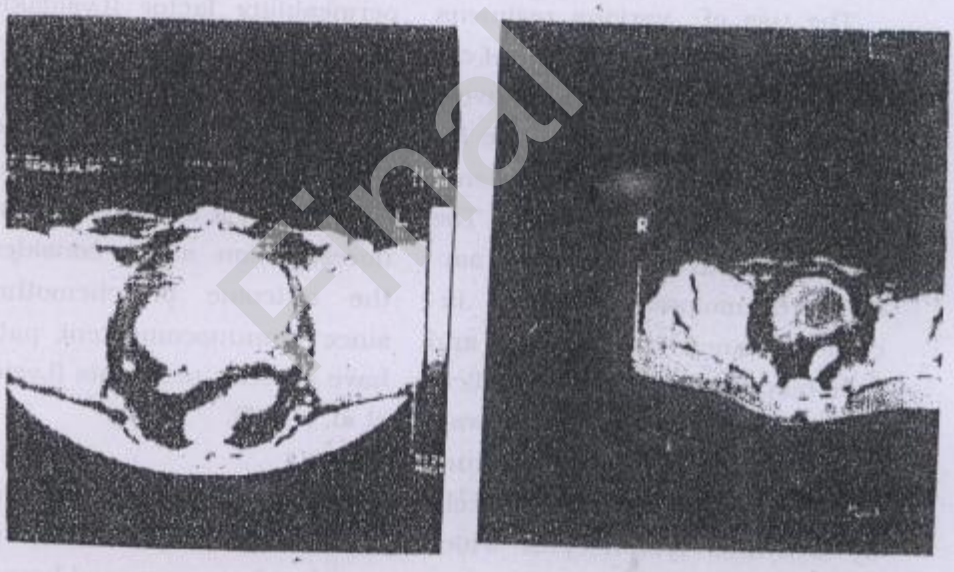

Fig. 3 : Malignant bladder tumour of stage T_{4b} N_1 M_0
a- Before treatment b- After treatment

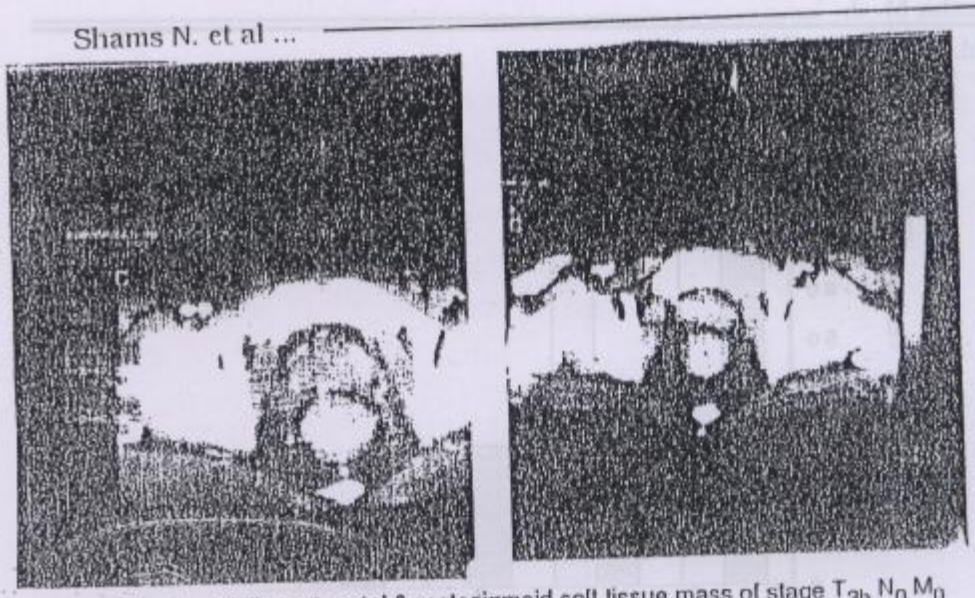

Fig. 4 : Malignant rectal & rectosigmoid soft tissue mass of stage T_{3b} N_0 M_0

a- Before treatment b- After treatment

Discussion

The use of various regimens followed by surgery as a sort of cytoreduction has been widely investigated to improve the chance for resectability & outcome of patients with locally advanced disease. The theoretical advantages of intraarterial chemoimmunotherapy include enhanced resectability and radioresponsiveness and eradication of micrometastasis (Fujiwaki et al., 1997). The direct immune attack to antigenic tumour cells by activated lymphocytes which also increase the vascular permeability by the secretion of vasoactive lymphokines as lymph node permeability factor and vascular permeability factor (Lygidakis et al., (1998). Moreover, chemotherapy caused profound depression of T-cell number & this reduction gradually and slowly recovers. That s why, it is likely that immunorestoration alters considerably the outcome of chemotherapy since immunocompetent patients have a better prognosis (Lygidakis et al, (1995).

The administration of chemotherapeutic drugs into the pelvic area has been proposed because it increases the local drug concentration at the tumour level and re-

duces the systemic toxicity (Edward et al., 1994) with high rates of immediate clinical and histological response (Kohno et al., 1993 and Patton et al., 1991) . Augmenting the effect of chemotherapy by immunotherapy has altered greatly the response because the injected lymphocytes are 100 times more effectivein therapeutic potency after expansion in IL-2 rather than the increase in number as evidenced by chromium 51 release microcytotoxicity assay (Jacobes et al., 1986).

In this study, the overall response rate was 82% and the remaining 5 cases showed stable disease, the difference was statistically significant $P < 0.01$, this finding is comparable to that reported by Lygidakis et al., (1998) (89.3%) . The response rates varied from 33%-41% partial response with intraarterial chemotherapy alone in cancer cervix (Carlson et al., 1981 and Sueyama et al., 1995) to 60% in cancer bladder (Sternbergh et al., 1981). On the other hand, some reports experienced that no superiority to intraarterial than systemic chemotherapy with no difference in plasma concentration of chemotherapy in renal or tumour content (Bielack et al., 1989 and Eilber et al., 1997).

In this study we treated 28 patients with locoregional immunotherapy and observed a remarkable response rate (82%) among the subjects even without the benefit of a respective procedure. This reinforces the belief that the patients immune response when stimulated is capable of controlling established tumour and, possibly, preventing further metastatic spread (Lygidakis et al., 1995). It has been established since 1990 that administered IL-2 results in the proliferation of macrophages, monocytes, and natural killer cells, (Lygidbakis et al., (1993) and the cytolitic conversion of tumour infiltrated lymhocytes against solid tumour cells (Lygidakis et al., 1998). So, the twofold benefit of a combination immunochemotherapy in addressing tumour burden and host response augmentation. first, induction of anatomic locoregional vascular disruptions via the venous phase of the transarterial administration route, which leads to tumour

necrosis and interruption of other possible micrometastasis, and, second, locoregional transfer of both cellular and humoral elements lead to further regional blood supply alterations and activation of lymphokine activated killer (LAK) cells (Lygidakis et al., 1998) , these beneficial effects are at work in the present study to explain the 82% favorable response to this treatment regimen.

In this study the OKT_3, OKT4 and ratio of OKT_4/OKT_8 were significantly increased in patients with locally advanced malignancy before treatment as compared to normal individuals values, stimulation caused by treatment had a significant effect on OKT_3 and OKT_4 production causing an increase in levels, indicating improve immune status after LAK infusion, these results are similar to that obtained by Lygidakis (Lygidakis et al., 1998). Complications secondary to the treatment regimen used as nausea & vomiting, diarrhae, haematuria, anaemia, leucopenia, thrombocytopenia and mucositis were acceptable and did not lead to any morbid consequences, these results are similar to that obtained by (Lygidakis et al., 1998). No episodes of catheter related blood stream infection due to strict sterile control, this data cope with that reports by (Raad et al., 1998) . Tearing of the arterial wall, inadvertent dislodgement and connection interruption between the infusion pump system and arterial catheter can be avoided with the placement of a catheter port in the subcutaneous tissue .

Conclusion

The intraarterial chemoimmunotherapy by regional perfusion employed in this study was well tolerated with a high overall response rate and acceptabe complications .So locoregional chemoimmunotherapy provides a palliation for most patients with locally advanced pelvic malignancies and may increase the respectability and improve tumour control in patients amenable for resection.

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