Local Continuous High Dose Interleukin 2: A New Therapeutic Model for the Treatment of Advanced Bladder Carcinoma

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ABSTRACT

Because efficient i.v. administration of interleukin 2 (IL-2) in tumor patients leads to severe cardiopulmonary side effects, we developed a new form of IL-2 application that allows activation of all IL-2-responsive immune cells directly at the tumor site. In five patients with T,N,M transitional cell carcinoma of the bladder, we used high-dose, continuous IL-2 perfusion of the bladder (1000 units/ml, 2 ml/min, 24 h/day), for 5 days after incomplete transurethral resection of the tumor. The total dose, 15 million units, was repeated after 4–12 weeks. None of our patients showed any evidence of side effects. In blood and especially in urine, the number of eosinophil leukocytes was remarkably increased. Eosinophils were attached to the tumor cells and degranulated on them. Before treatment, no cells positive for IL-2 receptors were found in urine, and only 0–3% of lymphocytes positive for IL-2 receptors were detected in blood. After the first cycle and especially after the second, a distinct increase in positive cells up to 17% occurred. One patient had a complete histologically confirmed remission and is free of tumor 6 months after therapy. Another patient died of a vertebral metastasis 8 weeks after the first (and only) therapeutic cycle.

Local administration of a high dose of IL-2 induces local and systemic immunopotentiation diagnosed by activation marker analysis without any side effects and can be used in inoperable transitional cell carcinoma of the bladder. The optimal dosage still needs to be defined. Bladder carcinoma is an ideal model that offers a unique chance to use urine cytology to study the cooperation and activation of immune cells at the tumor site.

INTRODUCTION

IL-2 is a potent modulator of immune response that has been reported to induce tumor regression in cancer patients (1–4). Incubation of unseparated lymphocytes in IL-2 in both mice and humans has led to the development of cells that destroy autologous and allogenic fresh tumor tissue. IL-2 supports growth of and boosts the activity of T-lymphocytes, B-lymphocytes, natural killer cells, and cytotoxic cells (5, 6). Tumor toxicity induced by IL-2 is correlated with dosage (7, 8) and time of incubation (9, 10). However, systemic i.v. administration of IL-2 in a dosage effective for treatment of cancer patients causes severe side effects, such as extensive fluid retention and life-threatening cardiopulmonary stress (1–3).

The efficacy and side effects of continuous local application of high-dose IL-2 have not been reported. The aim of our study was to prove the assumption that application of IL-2 at high and constant concentrations at a tumor site produces fewer side effects and activates tumor-infiltrating lymphocytes and other IL-2-responsive cells that might be involved in an antitumor response directly at the tumor site.

Deep invasive bladder carcinoma seems to be an ideal target for such an approach, because IL-2 is easy to apply there and has no definitive therapeutic regimen, such as chemotherapy, radiotherapy, or surgery, is available. Furthermore, bladder carcinoma is a tumor in which immune defense mechanisms have been shown to affect the course of the disease. Activation of the immune response is believed to be responsible for the therapeutic effects of Bacillus Calmette-Guérin (11, 12). Lymphocytes from transitional cell carcinoma patients are more likely than a variety of control cells to kill bladder tumor cells (13, 14).

MATERIALS AND METHODS

Patients and Treatment Schedule. Our subjects were five patients (four males and one female), 65–76 years old, with deep invasive transitional cell carcinoma, T,GII/III (Table 1). Informed consent was given by each patient. The study was proved by the Institutional Review Board of the Ärztekammer, Hamburg, West Germany, and the Ethik-Komitee of the Klinikum Steglitz Freie Universität Berlin, Berlin, West Germany.

Diagnosis was confirmed by histological examination after incomplete TUR. The bulk of the exophytic solid tumors was removed by incomplete TUR, except in patient 2. In all the patients, multiple specimens taken from the tumor ground at the end of TUR were found to contain cancer tissue. Two-handed examination with the patients anesthetized revealed a fixation of the bladder of each patient. Bone scanning, computed tomographic scanning, and chest X-ray were used to exclude patients with metastatic disease. Bladder perfusion started 2–8 days after TUR. n-IL-2 (provided by Biotest, Frankfurt, West Germany) was given in a continuous 24-h bladder perfusion at 2 ml/min and 1000 units/ml for 5 days. The bladder outflow tube was raised, e.g., 20 cm H2O, to distend the bladder wall without causing discomfort. Thus, each patient received 15 liters of n-IL-2 solution during the 5 days with a total dose of 15 million units. All patients were kept strict at bedrest for the 5 days and were given low dose heparin (100 units/kg every 12 h s.c.). The 5-day cycle of n-IL-2 bladder perfusion was repeated 4–12 weeks later, after another incomplete TUR. None of the patients had received chemotherapy or radiation therapy before the study. Before the perfusion (day 0) and immediately after it (day 5), blood and urine samples were collected; urine day 5 consisted of 50 ml, collected in the first 2 h after the IL-2 perfusion was finished. The samples were tested as described below.

Urine. Urine that had been subjected to cytocentrifugation was stained with the method of Pappenheim, May-Grünwald-Giemsa for cytological analysis and stained with a monoclonal IL-2 receptor antibody (IgGl) (Biotest, Frankfurt, West Germany) for detection of the 55,000-Da chain of the receptor by indirect immunocytochemistry. Lymphoprep preparations were fixed in acetone for 10 min and incubated for 30 min with 100 μl of 1:20 diluted IL-2-receptor antibody or 100 μl of a mouse monoclonal antibody of another specificity, but of the same isotype (IgGl) which served as a control to detect nonspecific binding. Each specimen was washed twice with 0.1 M Tris buffer at pH 8.7 and then incubated with alkaline phosphatase-conjugated goat anti-mouse IgG as a second antibody for 30 min with 100 μl of 1:20 diluted IL-2-receptor antibody or 100 μl of a mouse monoclonal antibody of another specificity, but of the same isotype (IgGl) which served as a control to detect nonspecific binding. Each specimen was washed twice with 0.1 M Tris buffer at pH 8.7 and then incubated with alkaline phosphatase-conjugated goat anti-mouse IgG as a second antibody for 30 min. The specimen was then washed twice with Tris buffer and incubated again with an alkaline phosphatase-conjugated swine anti-goat IgG for another 30 min. Endogenous alkaline phosphatase reaction was blocked with levamisole. The alkaline phosphatase activity was demonstrated with naphthol AS-BI phosphate. The cell preparations were counterstained with hemalum and mounted.

Blood. Blood cells from heparinized blood were stained with the method of Pappenheim, May-Grünwald-Giemsa. Heparinized blood was depleted of granulocytes and erythrocytes with Ficol-Hypaque density fractionation and depleted of macrophages with a consecutive plastic adherence procedure. Centrifuged preparations from the remaining cells were stained with a monoclonal IL-2-receptor antibody.
as described above. The percentage of stained cells was calculated. Creatinine, bilirubin, sodium, albumin, erthocytes, lymphocytes, leukocytes, thrombocytes, hemoglobin, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, and γ-glutamyltransferase were measured at days 0, 3, and 5 of therapy.

Natural IL-2. Natural human interleukin 2 was produced from phytohemagglutinin-stimulated, pooled, freshly donated human mononuclear cells. The product was highly purified and free of lectins and endotoxins. It was tested for the absence of several lymphokine activities: interleukin 1, interleukin 3, γ-interferon, macrophage-activating factor, leukocyte-inhibiting factor, granulocyte-inhibiting factor, macrophage-inhibiting factor, granulocyte-macrophage colony-stimulating factor, lymphotoxin, mitogenic factor, and granulocyte-chemiluminescence-activating factor. The specific activity of n-IL-2 was IO7 units/mg of protein. n-IL-2 consisted of a 16,000-Da glycosylated IL-2, a 15,500-Da glycosylated IL-2, and a 14,000-Da nonglycosylated IL-2 at a proportion of 1:1:1. The pattern of glycosylation corresponds to that of natural human IL-2.

RESULTS

Side Effects

No local or systemic side effects were observed in any of the patients. Serum and blood parameters monitored at days 0, 3, and 5 were normal, except for a slight increase in GPT to 33 units/liter and 30 units/liter (normal up to 28 units/liter) in patients 1 and 3, respectively, were positive (Fig. 4; Table 2). After the first cycle and especially after the second cycle, an increase in urinary receptor-positive cells was found (Table 2; Fig. 3).

Blood Cytology. The number of eosinophil leukocytes in blood increased in the first and second cycles in all patients except 2 (Table 2). Patient 5 demonstrated a distinct increase after the first cycle, but not after the second. He had remarkable urinary eosinophilia, however. Patients 1 and 3 with previously decreased blood lymphocyte counts had increased absolute lymphocyte counts after therapy. Patient 2, whose lymphocyte count was previously normal, had a lower count after therapy. Patients 4 and 5 remained within normal range of absolute lymphocyte counts during therapy.

Blood Immunocytochemistry and IL-2 Receptor Antibody. Before therapy, 0–3% of lymphocytes were positive for IL-2 receptor. After the first and second cycle of therapy, 2–5% and 9–17% of the lymphocytes, respectively, were positive (Fig. 4; Table 2).

Clinical Results

Patient 5 had a complete histologically verified remission and was free of tumor 6 months after therapy. Patient 2 died of vertebral metastases 8 weeks after the first and only therapeutic cycle. Partial remission is difficult to verify, because incomplete TUR before IL-2 therapy might contribute to tumor reduction.

Anti-IL-2 antibodies were not detectable in any of the patients 8 weeks after therapy (according to tests kindly performed by Dr. Schwuléra, Biotest Serum Institute).

DISCUSSION

We have shown that continuous high-dose application of IL-2 at a tumor site is tolerated without side effects. Even patients with an increased creatinine (patients 2 and 3) did not suffer deterioration of renal function. IL-2 acts as a local hormone, and high-dose local application should be preferred whenever possible. Even though n-IL-2 solution that perfuses the bladder is diluted with urine, a constant concentration of about 750 units/ml can be achieved at the tumor site that is not possible with i.v. infusion or with any other approach, because IL-2 is cleared from blood rapidly.

The finding in vitro that lymphokine-activated killer cells arise not earlier than the third day of incubation (2, 6) was the
Table 2  IL-2 receptor-positive cells and eosinophil leukocytes before (day 0) and after (day 5) the first and second cycle of IL-2 bladder perfusion

<table>
<thead>
<tr>
<th>Patients</th>
<th>Cycle</th>
<th>Day 0</th>
<th>Day 5</th>
<th>% of IL-2 receptor-positive cells in the blood</th>
<th>% of eosinophil leukocytes of total leukocytes in urine</th>
<th>Absolute count of eosinophil leukocytes in blood (x10⁶/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1st</td>
<td>0</td>
<td>+*</td>
<td>1</td>
<td>1</td>
<td>0.59</td>
</tr>
<tr>
<td>1</td>
<td>2nd</td>
<td>0</td>
<td>+</td>
<td>2</td>
<td>2</td>
<td>0.79</td>
</tr>
<tr>
<td>2</td>
<td>1st</td>
<td>0</td>
<td>+</td>
<td>2</td>
<td>2</td>
<td>0.16</td>
</tr>
<tr>
<td>3</td>
<td>1st</td>
<td>0</td>
<td>+</td>
<td>2</td>
<td>2</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>2nd</td>
<td>0</td>
<td>++</td>
<td>2</td>
<td>9</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>1st</td>
<td>0</td>
<td>++</td>
<td>2</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>2nd</td>
<td>(+)</td>
<td>++</td>
<td>0</td>
<td>13</td>
<td>1.39</td>
</tr>
<tr>
<td>5</td>
<td>1st</td>
<td>0</td>
<td>+</td>
<td>3</td>
<td>4</td>
<td>0.22</td>
</tr>
<tr>
<td>5</td>
<td>2nd</td>
<td>(+)</td>
<td>++</td>
<td>2</td>
<td>14</td>
<td>0.34</td>
</tr>
</tbody>
</table>

* (+), few positive cells; +, some positive cells; ++, many positive cells. Quantification of IL-2 receptor-positive cells in the urine was only roughly possible because the differentiation between lymphocytes and some tumor cells is sometimes not possible Example for ++ s (Fig. 3).

Fig. 1. Urinary cytology of patient 4 after the first cycle of continuous n-IL-2 treatment. Note degranulation and attachment of eosinophil leukocytes to bladder carcinoma cells. Pappenheim stain, × 400.

reason for the 5-day continuous treatment. This is in accord with our finding that an increase in cells positive for IL-2 receptor in the urine of our patients was not found before the third day of n-IL-2 therapy. We believe that continuous 24-h therapy for 5 days is important. Heparin should be given prophylactically to the immobilized patients.

After high-dose local IL-2 application in the bladder, a significant number of cells positive for IL-2-receptors could be seen after the fifth day of treatment. Positive cells could not be demonstrated in the urine of tumor patients before the first treatment cycle. The demonstrated increase in positive cells, especially after the second cycle, might represent not only T-lymphocytes, but also B-lymphocytes and macrophages. The exact nature of the positive cells must be studied further with double staining methods.

IL-2 receptors identified by the IL-2 receptor antibody appear later during lymphokine-activated killer cell activation in parallel with the expression of cytotoxic activity (15). IL-2 receptor expression is thought to be a requirement before IL-2-driven expansion of lymphoid cells.

Another reason for local IL-2 therapy is the observation that cytotoxicity is related to tumor stage in bladder carcinoma (13, 14). Our therapeutic model offers a chance to improve the known cytotoxicity by activating tumor-infiltrating T-lymphocytes, as well as other cells that respond to IL-2, such as B-lymphocytes, natural killer cells, and macrophages, which can also play an important role in the antitumor response.

The human natural IL-2 used in our study consists of two glycosylated molecular species and one nonglycosylated molecular species. Recombinant IL-2 consists of only a single nonglycosylated species. In vitro tests, cloning efficiencies of n-IL-2 proved superior to those of recombinant IL-2 (16). Further studies must be conducted to assess whether recombinant IL-2 will be tolerated as well and whether it has comparable immunopotentiating effects.

Mononuclear cells positive for IL-2 receptor in the blood were moderately increased after the first and markedly increased after the second cycle, which indicates that repeated stimulation results in a greater immunopotentiating effect. Normal persons do not have more than about 5% IL-2 receptor positive cells in peripheral blood, and most have below the detectable limit, which is approximately 1% (17). Our tumor patients had a maximum of 3% positive cells in peripheral blood before therapy. An increase up to 17% after the second cycle showed a distinct systemic effect, which could be a result of the spreading of activated lymphocytes from the tumor site.
rather than a result of the resorption of IL-2. Compared with the effects of i.v. administration, 17% positive cells is rather high. Lotze et al. (17) reported on a patient with melanoma who had 17.9% IL-2 receptor-positive cells after 2 weeks of continuous i.v. infusion at 1000 units/kg/h.

The penetration of IL-2 into tumor tissue is not known. To improve access of n-IL-2 to tumor-infiltrating leukocytes and to reduce the size of the tumor, we performed incomplete TUR on patients 3 to 8 days before starting n-IL-2 perfusion.

One of our most impressive findings was the occurrence of massive concentrations of urinary and blood eosinophils during IL-2 perfusions. Eosinophilia in blood of IL-2-treated patients is well known and has been considered to be a side effect of IL-2 therapy (1–4). The urinary and blood eosinophilia that we found in IL-2-treated patients is most probably related to T-cell-dependent regulation of eosinophilia.

In contrast with neutrophilic leukocytosis, increased eosinophil production in mice requires the participation of T-cells. Thymus-deprived mice are unable to react with eosinophilia to an appropriate stimulus, but their ability to mount a neutrophilic leukocytosis is unimpaired (18).

It has been suggested that leukocytes can stimulate bone marrow eosinopoiesis by releasing a diffusible agent or play a part in the elaboration of a factor chemotactic to eosinophils (18). Another group found that priming mice with tetanus toxoid induces formation of both T and B memory cells. Selective removal of B-lymphocytes did not impair the capacity of surviving T-lymphocytes to transfer a secondary eosinophil...
response while it abolished the capacity for transfer of secondary humoral antitoxin production. The authors concluded that T memory cells are involved in both the secondary eosinophilic response and the antitoxin response, whereas B memory cells appear to be involved only in the transfer of the secondary humoral response (19). In addition to their well-described participation in antiparasitic events, eosinophil participation in tumoricidal mechanisms has been suggested (20). Others have shown that eosinophil major basic protein is toxic to murine ascites tumor cells (21). Relatively low concentrations of eosinophil peroxidase were found to enhance mast cell tumoricidal activity in the presence of hydrogen peroxide and a halide (22). In this context, there is evidence that contact between the surface of human granulocytes and the surface of the target cell schistosomula results in release of cellular hydrogen peroxide and iodination. The generation of hydrogen peroxide and its interaction with peroxidase appear to be crucial in effecting granulocyte-mediated parasite toxicity (23). It is not known whether that mechanism in other target cells, especially tumor cells, is important. In the urinary sediment of our IL-2-treated patients, we found not only an increase in the number of eosinophils but also a close attachment of many of them to tumor cells. The eosinophils seem to accumulate around the tumor cells in vivo and deposit granular contents on them (Figs. 1 and 2). Attachment of eosinophils and degranulation as shown in these figures is in our experience not due to cytocentrifugation artifact. These changes could reflect the above-mentioned IL-2-initiated cooperation of the different cells of the immune system against bladder carcinoma. We think that it is no longer acceptable to call eosinophilia a side effect; it should be considered part of an effector mechanism in which T-cells and possibly primed memory cells are involved.

Urine from untreated patients with bladder carcinoma does not show the remarkable eosinophilia that was seen after IL-2 treatment, and urinary eosinophilia to that extent was never observed in patients with urinary tract infection, benign prostatic hypertrophy, and prostatic carcinoma. The effect seems to be rather specific for patients treated with IL-2.

Patient 5 had a complete tumor remission and had the highest urinary concentration of eosinophilic leukocytes after the first and second cycles. However, the blood level concentration of eosinophilic leukocytes after the second cycle was normal. A locally high concentration of eosinophilic leukocytes seems to be possible without an increase in systemic concentrations. Whether eosinophils are consumed after repeated cycles remains to be evaluated.

Patient 2, who died of previously unknown vertebral metastases, did not respond to IL-2 therapy by developing eosinophilia or IL-2-receptor-positive cells in blood and only a few were seen in the urine. That patient did not have a TUR before IL-2 treatment. We do not know whether prior TUR treatment is essential or whether patients with metastatic disease respond differently from patients who macroscopically are free of metastasis. We also do not know whether patients can be classified by other means as responders and nonresponders to that therapeutic approach. How often the cycles have to be repeated and the optimal time between cycles remains to be determined. Our therapeutic approach is, however, promising in patients who have advanced disease and in whom no alternative therapy is possible because of nonoperability or the danger of side effects.

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IMMUNOTHERAPY WITH INTERLEUKIN 2 IN BLADDER CARCINOMA

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