Locoregional IL-2 low dose applications for gastrointestinal tumors

Zachary Krastev, V Koltchakov, R Tomova, S Deredjian, A Alexiev, D Popov, B Tomov, Jan-Willem Koten, John Jacobs, Willem Den Otter

AIM: To explore the feasibility of local interleukin 2 (IL-2) in patients with different forms of abdominal cancer. This required experimentation with the time interval between IL-2 applications and the methods of application.

METHODS: Sixteen patients with stages III and IV of gastrointestinal malignancies (primary or metastatic) who were admitted to our Department of Gastroenterology were treated with locoregionally applied IL-2 in low doses.

RESULTS: No major problems applying locoregional IL-2 were encountered. In 6 out of 16 patients, a modest but clinically worthwhile improvement was obtained. Adverse effects were minimal. The therapeutic scheme was well tolerated, even in patients in a poor condition.

CONCLUSION: This study demonstrates the feasibility of low dose locoregional IL-2 application in advanced abdominal cancer. Local IL-2 therapy gives only negligible adverse effects. The results suggest that it is important to apply intratumorally. Local IL-2 may be given adjunct to standard therapeutic regimes and does not imply complex surgical interventions. These initial results are encouraging.

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INTRODUCTION

Treatment of cancer with intratumoral or peritumoral IL-2 application is very effective in mice with e.g. transplanted lymphoma[1] and colon carcinoma[2], and in guinea pigs with transplanted liver carcinoma[3]. In veterinary practice, it is also very effective in bovine ocular squamous cell carcinoma[4] and in equine sarcomas (fibro-epithelial tumors)[5]. Finally, it is effective in human patients with recurrent superficial bladder carcinoma following transurethral tumor resection[6], in patients with advanced nasopharyngeal carcinoma[7], and in patients with lung metastases of renal cell carcinoma[8]. Obviously, local IL-2 treatment does not cure all tumors. That is, in an experiment or a trial, usually only 50-70% of animals with large, metastasized tumors are cured or show complete tumor regression, whereas some tumors like EL4 lymphoma and MOT-carcinoma are not sensitive[9].

A well-known effect of IL-2 is vascular leakage. This side effect severely limits systemic application of IL-2. However, on a local level, this vascular leakage seems to be crucial for the therapeutic effect[2,12,13]. Locally applied IL-2 induces local vascular leakage and local edema, which results in massive tumor necrosis, a characteristic feature in most experimental animals after 5-20 d of local IL-2 treatment. Locally applied IL-2 also induces angiogenesis and attracts numerous macrophages and lymphocytes that invade the tumor debris and boost specific immunity against the tumor[14]. Animals that are cured from a transplanted tumor are immune to this tumor[1-3,13], suggesting that an immune response is essential for cure.

Recently we have successfully applied local IL-2 therapy to a patient with hepatocellular carcinoma[10] and to a patient with mesothelioma[11]. These results justified to proceed with local IL-2 therapy. In the present study, we have applied locoregional IL-2 therapy in 16 cancer patients with gastrointestinal malignancies (primary or metastatic) who were hospitalized in the University Gastroenterology Department in Sofia, Bulgaria. No alternative treatment was available for these patients. Our primary aim was to study the feasibility of locoregional IL-2 application in advanced abdominal cancer. Secondly, we wanted to establish whether clinical improvement could be obtained with this treatment. We present the results of local IL-2 treatment of patients with a variety of advanced abdominal cancers.

MATERIALS AND METHODS

Patients

All the 16 patients were admitted to the Clinic of Gastroenterology for primary or metastatic gastrointestinal cancer. All patients who were admitted to this IL-2 trial were advanced cases with progressive disease (PD, stages III and IV), either inoperable or not fit for standard chemo-
radiotherapy at the time of admission. Always a clinical oncologist was consulted to exclude that further radio- or chemotherapy was still a valid option. In all cases there was a histopathological diagnosis. Previous chemotherapy or radiotherapy was stopped at least 3 mo before IL-2 treatment. The patients were admitted to the trial, only if non-tumor-related serious conditions were absent such as heart, pulmonary, endocrine, or kidney disease. IL-2 was applied only following extensive information of the patients and with their full consent. All patients were treated in the period of 1999-2003. In Table 1 the characteristics of these patients are summarized.

**Interleukin-2**

Recombinant human IL-2 (rhIL-2; Proleukin; specific activity $18 \times 10^6$ IU/mg) from Chiron was used. RhIL-2 was dissolved according to the manufacturer’s instructions and, if necessary, further diluted with 1% albumin and 0.9% NaCl.

**Treatment**

Our primary intention was to apply IL-2 as close as possible to the tumor (primary or metastatic) and at the site of the antitumor immune reaction. Direct intra-peritoneal IL-2 was applied in carcinosis of the peritoneum. In patients W5, W7, and W8, IL-2 could be instilled directly into the malignant ascites. In patients W1, W2, W3, and M3 ‘ascites’ was artificially made by infusing between 500 and 2 000 mL 0.9% NaCl into the peritoneal cavity. Thereafter IL-2 was injected into this ‘ascites’, in order to ensure an intra-peritoneal spread of IL-2.

In hepatic tumors (primary or metastatic), IL-2 was injected directly into the tumor using a fine needle under ultrasound control or via the hepatic artery under X-ray control. In colon cancer patients with unresected primary tumor, IL-2 was endoscopically applied directly into the tumor. When it was possible, IL-2 was also directly injected into accessible peritoneal tumors.

We applied IL-2 weekly and monthly for 2 mo. In patients with weekly applications, IL-2 was injected every week in 7-d interval and in patients with monthly applications IL-2 was applied every month in 30-d intervals. This was followed by application of IL-2 at 3-mo intervals.

At the beginning of the treatment, when it was possible, we artificially induced necrosis in order to stimulate the immune reactivity\(^{[18,19]}\). For this purpose, 10 d before the start of IL-2 treatment, we applied single procedures of local ablation-percutaneous ethanol injection in five patients with liver tumors (primary or metastatic) and in one patient with unresected primary colorectal cancer-endoscopic ablation.

**Ethical committee**

The treatment protocol was accepted by the ethical committee of our hospital in Sofia.

**Effect of treatment**

(1) The effect of therapy was assessed as follows: complete response (CR: disappearance of all tumors and signs of disease); partial response (PR: reduction >50% in the sum of the products of perpendicular diameters of bidimensionally measurable disease or for unidimensionally measurable disease a reduction of $\geq 50\%$ in linear tumor measurement compared to those prior to treatment); stable disease (SD: <50% reduction, or an increase of <25% in the sum of the products of perpendicular diameters of bidimensionally measurable disease or in linear tumor measurement for

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**Table 1 Patients’ characteristics**

| No. | Sex | Primary tumor          | Metastases                        | Neoplastic ascites | Route and dose of IL-2 application | Necrosis induction |
|-----|-----|------------------------|----------------------------------|--------------------|------------------------------------|-------------------|
| W1  | M   | Pancreatic Ca          | Liver, abdominal lymph nodes     | -                  | 1.5 MIU i.p.                       |                   |
| W2  | F   | Stomach Ca             | Liver, abdominal lymph nodes     | -                  | 4.5 MIU i.p.                       |                   |
| W3  | F   | Colorectal Ca          | Liver, lung                       | -                  | 4.5 MIU i.p.+i.t.                  | Yes               |
| W4  | F   | Colorectal Ca          | Liver, abdominal lymph nodes     | -                  | 4.5 MIU i.t.                       | Yes               |
| W5  | M   | Colorectal Ca          | Peritoneum                        | +                  | 1.5 MIU i.p.+i.t.                  | -                 |
| W6  | F   | Ovarian Ca             | Lung, peritoneum, spleen          | +                  | 4.5 MIU i.t.                       |                   |
| W7  | F   | Mammary Ca             | Liver, lung, peritoneum           | +                  | 4.5 MIU i.p.                       |                   |
| W8  | M   | Hemangiosarcoma        | Liver, peritoneum, abdominal lymph nodes | *pleural effusion | 4.5 MIU i.p.+i.t.                  | -                 |

B. Once monthly local IL-2 application

| No. | Sex | Primary tumor          | Metastases                        | Neoplastic ascites | Route of IL-2 application | Necrosis induction |
|-----|-----|------------------------|----------------------------------|--------------------|---------------------------|-------------------|
| M1  | M   | HCC, liver cirrhosis   | -                                | -                  | 4.5/9 MIU i.t.            | -                 |
| M2  | M   | HCC, liver cirrhosis   | -                                | -                  | 4.5 MIU i.t.              | Yes               |
| M3  | M   | Abdominal mesothelioma | Peritoneum                        | -                  | 9 MIU i.t.+i.p.           | -                 |
| M4  | F   | Colorectal Ca          | Liver                            | -                  | 9 MIU i.t.                | Yes               |
| M5  | F   | Mammary Ca             | Liver, bone, skin                | -                  | 1.5 MIU i.t.              |                   |
| M6  | F   | Mammary Ca             | Liver                            | -                  | 4.5/9 MIU i.t.            | -                 |
| M7  | M   | HCC, liver cirrhosis   | -                                | -                  | 4.5 MIU i.t.              | Yes               |
| M8  | F   | HCC, liver cirrhosis   | -                                | -                  | 1.5 MIU i.t.              |                   |

Ca, carcinoma; HCC, hepatocellular carcinoma; i.p., intraperitoneal; i.t., intratumoral; W, weekly IL-2 application; M, monthly IL-2 application.
unidimensionally measurable disease compared to those prior to treatment with no new lesions for at least 4 wk; or PD (increase of $\geq 25\%$ in the sum of the products of perpendicular diameters of bidimensionally measurable disease or in linear tumor measurement for unidimensionally measurable disease compared to those prior to treatment/or the appearance of new lesions). Response to treatment was assessed with CT scan and ultrasound control; (2) IL-2 is thought to be effective in palliative therapy of neoplastic effusions\textsuperscript{[21]}. For this reason in patients with malignant ascites we also assessed the effect of our treatment on fluid accumulation according to the following criteria: if there was a complete disappearance of ascites with no further reaccumulation of fluid for more than 30 d, it was defined as CR; if the ascites decreased but there was still minimal asymptomatic fluid, not requiring further drainage within 30 d, it was a PR; if there was further accumulation of fluid requiring paracenteses within 30 d, it was considered as a failure. The patients were assessed by ultrasound control and number of paracenteses. Our criteria for evaluation are similar to those reported by Lissoni et al.\textsuperscript{[20]}, but we did not drain the ascitic fluid before IL-2 applications in order to enable better spread of IL-2. This assessment was performed 3 and 6 mo after the commencement of treatment. Separately we assessed the Karnofsky Index in patients in order to follow the changes in quality of life.

**RESULTS**

**Feasibility**

Application of IL-2 at the site of the tumor was feasible in all patients. No major obstacles were encountered. Locoregional IL-2 application in doses 1.5-9 MIU IL-2 did not cause undue discomfort, even in patients in poor general condition.

**Therapeutic effect**

The clinical effects of local IL-2 application are shown in Table 2. (1) After IL-2 treatment, most patients (12/16) had PD. One patient of the ‘weekly’ scheme (W4) and three patients of the “monthly” scheme (M2, M7, and M8) had SD. These four patients had PD at the start of therapy and stabilization of their disease is a success that was proved by their survival (Table 2). Patient W4 was cachectic at the first presentation. After IL-2 treatment, her condition improved (Karnofsky index of 50 became 80), she had SD for 7 mo. Thereafter liver metastases slowly progressed, liver function tests worsened, but the primary tumor in the colon did not progress. Her survival was 28 mo. Patient M2 showed SD for 8 mo. He was in a relatively good clinical condition and this remained so. Patient M7 had SD for more than 2 years (at the time of diagnosis of HCC he was decompensated, had cirrhosis Child C, and diabetes), and enjoyed an improved quality of life. This patient did not die from the tumor, but due to liver cirrhosis. Patient M8 had SD for 23 mo. At the time of writing this paper a progression of the tumor was registered, but she is still in good general condition. (2). Neoplastic ascites. All patients with malignant effusion were of the ‘weekly’ scheme. Two of them showed complete reduction of ascites (2/4) after IL-2 treatment, and the other two showed failure to IL-2 treatment. Responding patients were patients W5 and W8. In these two patients, IL-2 was initially applied directly into

**Table 2 Therapeutic effect after 3\textsuperscript{rd} mo and survival**

| No. | Sex | Primary tumor     | Clinical effect | Neoplastic effusion | Karnofsky before and after treatment (%) | \*Survival mo |
|-----|-----|------------------|-----------------|---------------------|----------------------------------------|-------------|
| A. Once weekly local IL-2 application |     |                  |                 |                                   |             |
| W1  | M   | Pancreatic Ca    | PD              | -                   | 30-30                                  | 4           |
| W2  | F   | Stomach Ca       | PD              | -                   | 80-60                                  | 3           |
| W3  | F   | Colorectal Ca    | PD              | -                   | 80-60                                  | 1           |
| W4  | F   | Colorectal Ca    | SD              | -                   | 50-80                                  | 28          |
| W5  | M   | Colorectal Ca    | PD              | Complete reduction  | 70-90                                  | 10          |
| W6  | F   | Ovarian Ca       | PD              | Failure             | 60-50                                  | 2           |
| W7  | F   | Mammary Ca       | PD              | Failure             | 60-40                                  | 1           |
| W8  | M   | Hemangiosarcoma  | PD              | Complete reduction  | 50-80                                  | 12          |
| B. Once monthly local IL-2 application |     |                  |                 |                                   |             |
| M1  | M   | HCC Liver cirrhosis | PD            | 70-70                | 4                                       |             |
| M2  | M   | HCC Liver cirrhosis | SD            | 80-80                | 24A                                     |             |
| M3  | M   | Abdominal mesothelioma | PD         | 80-50                | 6                                       |             |
| M4  | F   | Colorectal Ca    | PD              | 80-70                | L                                       |             |
| M5  | F   | Mammary Ca       | PD              | 40-40                | 1                                       |             |
| M6  | F   | Mammary Ca       | PD              | 80-80                | L                                       |             |
| M7  | M   | HCC Liver cirrhosis | SD            | 90-90                | 27                                      |             |
| M8  | F   | HCC Liver cirrhosis | SD            | 80-90                | 24A                                     |             |

1\ survival since the start of IL-2 therapy till death; W, weekly IL-2 application; M, monthly IL-2 application; A, alive; L, lost for follow-up; Ca, carcinoma; HCC, hepatocellular carcinoma; PD, progressive disease; SD, stable disease.
the neoplastic effusion, but with the reduction of the ascites, subsequent IL-2 applications were made into artificially made ascites. Patient W8 had complete reduction of ascites and significant reduction of the pleural effusion, though liver metastases progressed. The quality of life after IL-2 treatment had improved (Karnofsky index of 50% became 80%). Patient W5 had complete reduction of ascites and improved quality of life (Karnofsky of 70% became 90%) enabling him to resume his usual activities. (3) In conclusion, 6 out of 16 patients seemed to benefit more or less from IL-2 application at the site of the tumor. Three out of eight patients of the ‘weekly’ scheme improved -one had SD and two had reduction of ascites, and also 3/8 patients of the “monthly” scheme showed SD. Therapeutic results did not differ between the ‘weekly’ and the ‘monthly’ treatment schedule. Comparison with historical controls in our study was not possible due to the heterogeneity of our patients. In future larger prospective trial should be done.

Adverse effects
Adverse effects were minimal. The therapeutic scheme was well tolerated, even in patients in a poor condition. Often moderate fever was observed, though rarely above 38.2 °C. A transient local skin rash was observed in one patient after intra-peritoneal IL-2 application.

DISCUSSION
This study shows that treatment of cancer, presented at a gastro-intestinal clinic, with locoregionally applied IL-2 in doses 1.5-9 MIU was feasible. No major problems arose. Locoregional IL-2 treatment induced a modest but worthwhile clinical gain: 6/16 patients benefited from this treatment. Four patients had SD for some time after IL-2 treatment and two had reduction of ascites. Four patients had an improved Karnofsky Index. Two patients lived considerably longer than was clinically expected (>24 and 27 mo).

Probably vascular leakage causing edema and subsequent tumor necrosis are indirect effects of local IL-2 application. IL-2 has pleiotropic immune effects in vivo and probably leads to macrophage reaction and homing of specific lymphocytes. Induction of tumor necrosis by irradiation[18] or Cisplatin[6,18] further enhances the therapeutic effect of following local IL-2 injections. Therefore, necrosis was induced in five of our patients. We applied only single procedure of local ablation, which cannot create significant necrosis, and therefore cannot lead to the therapeutic effect observed in our patients alone.

Whereas chemotherapy of cancer is usually connected with serious side effects such as extensive nausea and fatigue, leucopenia and related to this infections, such features were not observed in the patients treated with local IL-2 applications in these doses. Another advantage of this treatment is that IL-2 can usually be applied without complex surgical intervention. Further this treatment is cheap since only limited amounts of IL-2 are required.

Therapeutic gain might even be better if we can fine-tune our protocol. The results of this feasibility study thus warrant proceeding with a larger trial to further establish the effect of local IL-2 application in advanced gastro-intestinal cancer in combination with standard anti-cancer treatment.

In conclusion, this study demonstrates the feasibility of low dose locoregional IL-2 application in advanced abdominal cancer.

Local IL-2 application in patients with advanced GI malignancies led to modest but worthwhile clinical improvement in 6 out of 16 patients.

Important are the absence of adverse effects or serious discomfort, and the limited expenses.

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