Locoregional IL-2 Therapy in the Treatment of Colon Cancer. Cell-induced Lesions of a Murine Model

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Abstract. *Background: Local therapy with IL-2 may be very effective in the treatment of different forms of cancer. The aim of this study was to determine the effectiveness of IL-2 locoregional application in the treatment of colon cancer. Materials and Methods: Twenty eight syngenic BDIX rats were utilized in this study. The rats were divided into two groups of fourteen animals: group T (treatment) and group C (control). All rats of both groups were injected, under the splenic capsule, with T 10^7 DHD/K12/ TRb neoplastic cells. Then, within and around the site of the previous inoculation, the T group was injected with 1 ml of glucosate solutions + 0.1% albumin (BSA) containing 2.5x10^6 IU of IL-2 (Proleukin-Chiron), whereas the C group was injected with 1 ml of BSA alone. After three weeks, rats were sacrificed and the liver and spleen were removed. The following parameters were considered: volume and weight, neoplastic–non neoplastic tissue index of the spleen, mitotic index and vascular density of splenic and hepatic lesions. Results: All the studied parameters showed statistically significant differences in treated and untreated animals. Conclusion: This study of a murine model demonstrated that IL-2 locoregional therapy may be effective in the treatment of colon cancer.*

Surgical treatment is the primary therapy for solid tumors. However, a complementary therapy to surgery I necessary, particularly in an advanced tumor stage. Among complementary therapies, immunotherapy with interleukin (IL-2) seems to be the more promising. IL-2 as a therapeutic agent has been used in animal models as well as in human cancer therapy (1-6).

IL-2, the main growth factor of CD8+ lymphocytes and natural killer (NK) cells, has been evaluated in patients with metastatic colorectal carcinoma but the results have not been conclusive (5). Den Otter *et al.* reported in a letter that, while they had no experience with IL-2 local treatment in colorectal cancer, they believed that at least a proportion of patients with colorectal cancer could benefit from local IL-2 therapy (7). Since then, other studies have been published demonstrating the effectiveness of IL-2 therapy in gastrointestinal tumors (8-11).

The aim of this study in a murine model was to evaluate the effectiveness of IL-2 as an antiproliferative agent and in prevention of hepatic metastases when injected in sites that were previously inoculated with colorectal cancer cells.

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Materials and Methods

Twenty eight syngenic BDIX rats (Charles River Laboratoires, Lecco, Italy) were utilized for the study, carried out according to international principles for Biomedical Research on Animals (US Department of Health and Human Science, National Institutes of Health, 1985). Two groups, T (treatment) and C (control), of 14 rats each were formed. Under intraperitoneal anesthesia with Na-thiopental 1.25% at 40 mg/kg, a short midline laparotomy was performed in each rat of both groups and 0.5 ml of phosphate buffered saline (PBS) solution containing 10^7 DHD/K12/TRb neoplastic cells was injected under the spleen capsule, according to the technique used by Karube *et al.* (12). Rats in the T group were injected with 1 ml of glucosate solution +0.1% albumin (BSA) containing 2.5x10^6 IU of IL-2 (Proleukin, Chiron) within and around the previously inoculated site; rats in the C group were injected with 1 ml of BSA alone as placebo. After three weeks, rats were sacrificed and the liver and spleen were removed, weighed and fixed in formalin to evaluate the number, dimension and size (volume) of the cancerous lesions in the two groups and to perform a histological study. The following parameters were considered: weight, size, volume and neoplastic–non neoplastic tissue index of the spleen; spleen and hepatic mitotic index; peritoneal cancer index (PCI) according to a modified Sugarbaker's score (1= lesion of 1 mm; 2= from 1 to 5 mm; 3= up to 5 mm or confluent); vascular density (13).

The vascular density of the lesions developed in treated and untreated rats was analyzed in sections of spleen stained for factor VIII and CD31 using the peroxidase-antiperoxidase method (Blood

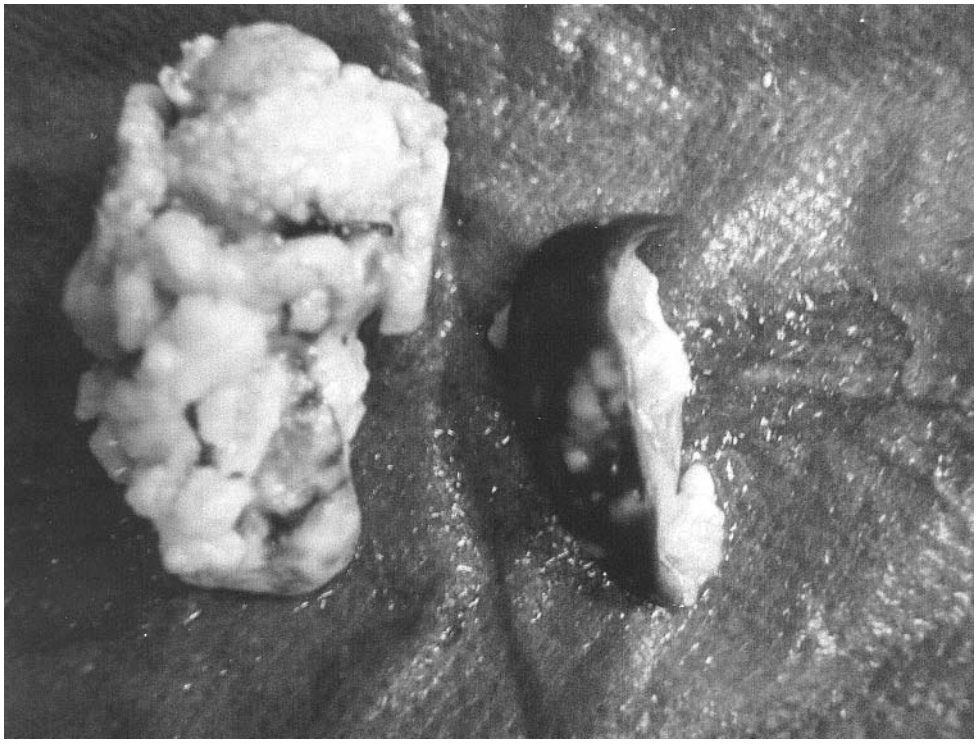

Figure 1. *A macroscopic image of dissected rat spleens. On the left, an untreated spleen demonstrates a complete substitution of the normal parenchyma with the neoplasia. Small and sporadic nodules can be seen in the IL-2-treated spleen on the right.*

Vessel Staining Kit Peroxidase System, Chemicon International, CA, USA); areas of 1 mm^2 were examined, containing seven adjacent microscopic fields (x400) each and only intralesional vessels were counted. In order to assess the statistical significance of the difference between the two groups of rats, the Mann-Whitney test was used. The test was performed as a two-tailed test. Two-sided p-values <0.05 were considered to indicate statistical significance. All statistical analysis was performed with SPSS statistical software version 11.5 (SPSS Inc., Chicago IL, USA).

Results

Inoculations of neoplastic cells and of IL-2 in the spleens were achieved in all rats. The dose utilized did not cause discomfort in any animal. After three weeks, the effectiveness of the IL-2 locoregional application was very clear. The macroscopic effects are evident in Figures 1 and 2, showing the remarkable difference of neoplastic growth in the two groups. All the parameters measured: volume and weight, neoplastic/non-neoplastic tissue index of the spleen, spleen and liver mitotic index and the vascular density of splenic and hepatic lesions showed statistically significant differences in treated and untreated animals; the histology of the spleen and the liver from an untreated animal (C group) is depicted in Figure 3, whereas the histology from an IL-2 treated animal (T group) in Figure 4.

Table I. *Results from the analysis of explanted organs*[a].

	Treated rats	Untreated Rats
Spleen volume*	2.20 (cm) (0.51)	4.55 (cm) (1.44)
Spleen weight**	2.39 (g) (0.39)	5.1 (g) (1.27)
Cancer/healthy tissue ratio in spleen*	0.35 (0.11)	0.59 (1.20)
Spleen mitotic index **	15.57 (6.28)	26.57 (4.70)
Liver mitotic index*	5.43 (6.80)	17.85 (4.45)
Liver metastasis vasal density**	73.08 (mm) (3.85)	187.09 (mm) (6.67)

[a]Values are means and standard deviations (SD). *$p<0.01$, **$p<0.001$ using the Mann-Whitney test.

Moreover, the peritoneal cancer index was 39 in untreated rats and ranged from 3 to 6 in treated animals (Figure 2). Table I confirms that the effectiveness of the therapy was indubitable in the murine model.

Discussion

Human colorectal cancer has a high incidence and is a major health problem in economically developed countries; moreover, at an advanced stage it has a poor prognosis after

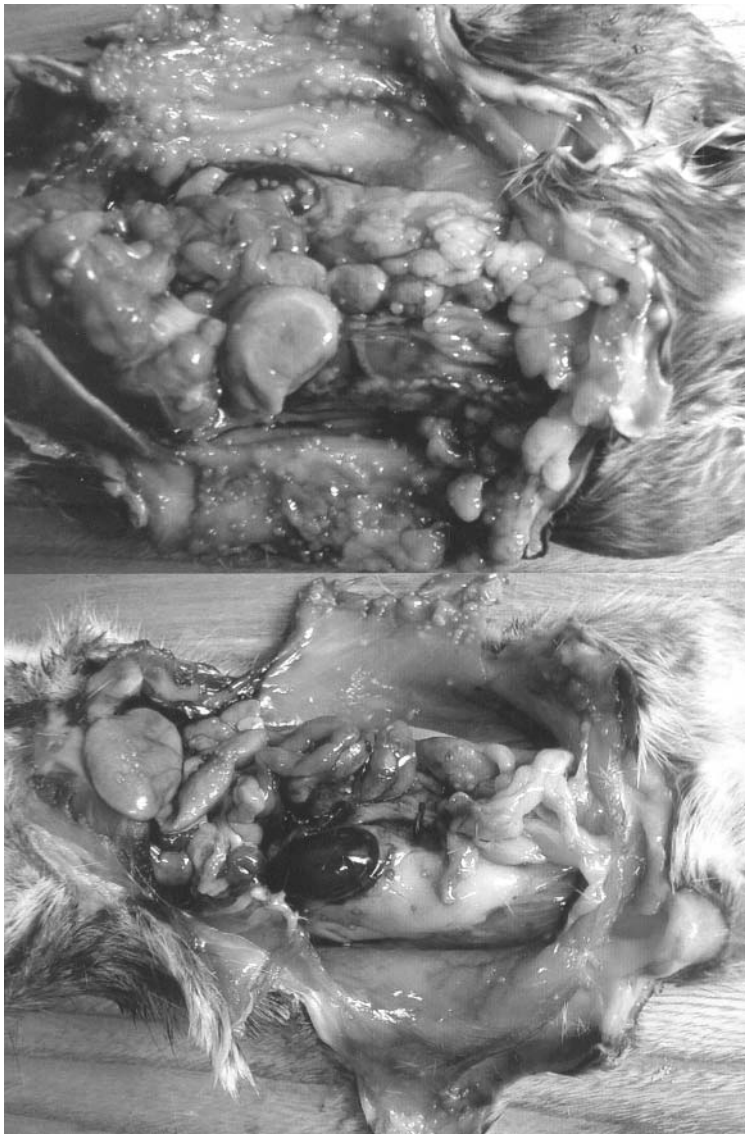

Figure 2. *An autoptic view of the abdominal cavities. The upper image depicts an untreated rat with diffuse peritoneal carcinomatosis and the lower, an IL-2-treated rat with some small sporadic lesions.*

surgery. As a result of its resistance to classical chemotherapeutic treatments, it produces rarely curable metastases and recurrences (1, 14, 15). New chemotherapeutic agents have been developed *e.g.* the topoisomerase 1 inhibitor, irinotecan, and a new platinum derivate, oxaliplatin. These agents constitute a second-line chemotherapy for patients who failed to respond to 5-FU chemotherapy. However, despite a significant improvement in the quality of life with this chemotherapy, the results in terms of survival remain very poor and a cure is exceptional for patients with evolutive metastatic colorectal cancer. Immunotherapy with IL-2, which increases immune responsiveness against the tumor, has proven effective in melanoma and renal cell carcinoma, probably because of their immunogenicity (16). Unfortunately, in colorectal cancer this therapy is deceiving, perhaps because of the poor imunogenicity of the cells (2, 17, 18). In fact, tumor cells may avoid the host's immune response by various means, such as a lack of antigen presentation and/or the absence of accessory molecules involved in cell adhesion. Rosenberg *et al.* (2) reported no conclusive results of IL-2 therapy in patients with metastatic colorectal carcinoma. The effect of local injection of IL-2, both experimentally and clinically (19-21), was reported to have good results,

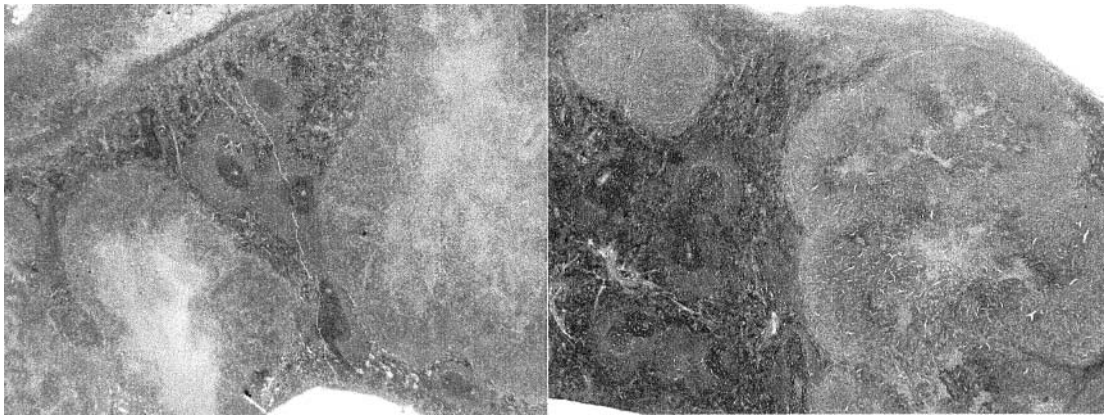

Figure 3. *Histology of the spleen and liver from an untreated animal (C group): multiple nodules of poorly differentiated neoplasia obscure the splenic parenchyma (on the left) and the liver (on the right). (H&E x15).*

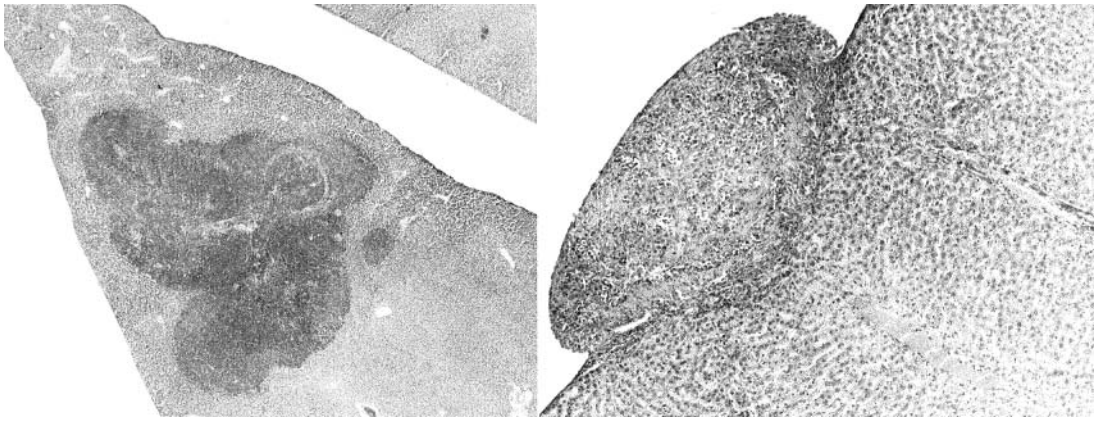

Figure 4. *Histology of the spleen and the liver from an IL-2 treated animal (T group): nodules of poorly differentiated neoplasia are smaller and sporadic in the splenic parenchyma (on the left) and a single subcapsular nodule is depicted on the right. (H&E x15).*

although these researchers admitted to having no experience with this therapy in colorectal cancer. However, they believed that patients affected by colorectal cancer could respond, at least in part, to IL-2 and proposed the injection of IL-2 at the site of the tumor intraoperatively or within two days after surgery in patients who undergo incomplete resection of a large tumor and/or metastases. Further reports confirm the usefulness of locoregional administration of IL-2 in treatment of gastrointestinal tumors (6, 8). Some authors emphasize the well known "vascular leakage" effect induced by local administration of IL-2 (19-21); moreover, IL-2 may induce a specific immunity against the tumor by activating large numbers of macrophages and lymphocytes (22-23). Our first aim was to experimentally evaluate the effectiveness of locally administered IL-2 against colorectal carcinoma cells in rats. The DHD/K12/TRb cell line we utilized is derived from a chemically induced BDIX rat metastatic colon carcinoma. The effects of IL-2 injected within and around the DHD/K12/TRb inoculated site of the spleen were very demonstrative. Comparing the variables of the T and C group using the Mann-Withney test, statistically significant differences were observed in spleen volume, spleen weight, neoplastic/non-neoplastic tissue index, mitotic index of the spleen and of the liver lesions and finally the vascular density of these lesions in the spleen, confirming the antiproliferative action of IL-2 in lesions from injected colorectal carcinoma cells of the rat.

Moreover, the low score of the PCI in treated rats (3-6) and the very low incidence of small distant lesions observed on Glisson capsules attested to the IL-2 ability of reducing distant metastases.

Conclusion

We may affirm that local administration of IL-2 at the site of inoculated colorectal carcinoma cells shows an antiproliferative effect and extensively reduces distant metastases in the rats.

References

1 Perrin P, Cassagnau E, Burg C, Patry Y, Vavasseur F, Harb J, Le Pendu J, Douillard JY, Galmiche JP, Bornet F and Meflah K: An interleukin 2/sodium butyrate combination as immunotherapy for rat coloncancer peritoneal carcinomatosis. Gastroenterology *107(6)*: 1697-1708, 1994.

2 Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, Seip Ca, Simpson CG and White DE: A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2alone. N Engl J Med *316(15)*: 889-897, 1987.

3 Urba WJ, Clark JW, Steis RG, Bookman MA, Smith JW 2nd, Beckner S, Maluish AE, Rossio JL, Rager H, Ortaldo JR and Ongo DL: Intraperitoneal lymphokine-activated killer cell/interleukin-2 therapy in patients with intra-abdominal cancer: immunologic considerations. J Natl Cancer Inst *81(8)*: 602-611, 1989.

4 Balemans LT, Mattijssen V, Steerenberg PA, Van Driel BE, De Mulder PH and and Den Otter W: Locoregional therapy with polyethylene-glycol-modified interleukin-2 of an intradermally growing hepatocellular carcinoma in the guinea pig induces T-cell-mediated antitumor activity. Cancer Immunol Immunother *37(1)*: 7-14, 1993.

5 Hihara J, Yamaguchi Y, Minami K, Noma K and Toge T: Down-regulation of IL-10 enhances the efficacy of locoregional immunotherapy using OK-432 against malignant effusion. Anticancer Res *19(2A)*: 1077-1084, 1999.

6 Yamaguchi Y, Satoh Y, Miyahara E, Noma K, Funakoshi M, Takashima I, Sawamura A and Toge T: Locoregional immunotherapy of malignant ascites by intraperitoneal-administration of OK-432 plus IL-2 in gastric cancer patients. Anticancer Res *15(5B)*: 2201-2206, 1995.

7 Den Otter W, Ziekmann PGPM, Koten JW and Lygidakis NJ: suggestions/requirements for a protocol to treat colorectal cancer with IL-2. Hepatogastroenterology *48(37)*: Letter to Editor, 2001.

8 Kusnierczyk H, Pajtasz-Piasecka E, Koten JW, Bijleveld C, Krawczyk K and Den Otter W: Further development of local IL-2 therapy of cancer: multiple *versus* single IL-2 treatment of transplanted murine colon carcinoma. Cancer Immunol Immunother 53(5): 445-452, 2004.

9 Romano F, Cesana G, Berselli M, Gaia Piacentini M, Caprotti R, Bovo G and Uggeri FR: Biological, histological, and clinical impact of preoperative IL-2 administration in radically operable gastric cancer patients. J Surg Oncol *88(4)*: 240-247, 2004.

10 Yamaguchi Y, Miyahara E, Ohshita A, Kawabuchi Y, Ohta K, Shimizu K, Minami K, Hihara J, Sawamura A and Toge T: Locoregional immunotherapy of malignant effusion from colorectal cancer using the streptococcal preparation OK-432 plus interleukin-2: induction of autologous tumor-reactive CD4+ Th1 killer lymphocytes. Br J Cancer *89(10)*: 1876-1884, 2003.

11 Krastev Z, Koltchakov V, Tomova R, Deredjian S, Alexiev A, Popov D, Tomov B, Koten JW, Jacobs J and Den Otter W: Locoregional IL-2 low dose applications for gastrointestinal tumors. World J Gastroenterol *11(35)*: 5525-5529, 2005.

12 Karube H, Masuda H, Hayashi S, Ishii Y and Nemoto N: Fatty liver suppressed the angiogenesis in liver metastatic lesions. Hepatogastroenterology *47(36)*: 1541-1545, 2000.

13 Jacquet P and Sugarbaker PH: Clinical research methodologies in diagnosis and staging of patients with peritoneal carcinomatosis. *In*: Peritoneal carcinomatosis: principles of management. Sugarbaker PH (ed). Boston, Kluwer Academic, pp. 359-374, 1996.

14 Boring CC, Squires TS and Tong T: Cancer Statistics. Cancer J Clin *42*: 19-38, 1992.

15 Moertel CG: Accomplishments in surgical adjuvant therapy for large bowel cancer. Cancer *70(5 Suppl)*: 1364-1371, 1992.

16 Huland E, Heinzer H and Huland H: Treatment of pulmonary metastatic renal-cell carcinoma in 116 patients using inhaled interleukin-2 (IL-2). Anticancer Res *19(4A)*: 2679-2683, 1999.

17 Beatty JD: Immunotherapy of colorectal cancer. Cancer *70(5 Suppl)*: 1425-1433, 1992.

18 Gazit Z, Weiss DW, Shouval D, Yechezkeli M, Schirrmacher V, Notter M, Walter J and Kedar E: Chemo-adoptive immunotherapy of nude mice implanted with human colorectal carcinoma and melanoma cell lines. Cancer Immunol Immunother *35(2)*: 135-144, 1992.

19 Jacobs JJ, Sparendam D and Den Otter W: Local interleukin 2 therapy is most effective against cancer when injected intratumourally. Cancer Immunol Immunother *54(7)*: 647-654, 2005.

20 Krastev Z, Koltchakov V, Vladov N, Popov D, Milev A, Koten JW and Den Otter W: A mesothelioma that is sensitive to locally applied IL-2. Cancer Immunol Immunother *50(4)*: 226-227, 2001.

21 Den Otter W, Balemans L, Battermann JJ, Bernsen MR, Cadee JA, Dobrowolski Z, Everse LA, Fiszer-Maliszewska L, Gavhumende R, De Groot JW, De Groot K, Hennink WE, Hill FW, Jurgenliemp-Schulz I, Klein WR, Koten JW, Maas RA, Steerenberg P, Stewart R and Zembala M: Local low-dose IL-2 therapy. Hepatogastroenterology *46(Suppl 1)*: 1280-1286, 1999.

22 Baselmans AH, Koten JW, Battermann JJ, Van Dijk JE and Den Otter W: The mechanism of regression of solid SL2 lymphosarcoma after local IL-2 therapy. Cancer Immunol Immunother *51(9)*: 492-498, 2002.

23 Van Es RJ, Baselmans AH, Koten JW, Van Dijk JE, Koole R and Den Otter W: Perilesional IL-2 treatment of a VX2 head-and-neck cancer model can induce a systemic anti-tumour activity. Anticancer Res *20(6B)*: 4163-4170, 2000.

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