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Regional Adoptive Immunotherapy with Interleukin-2 and Lymphokine-activated Killer (LAK) Cells for Liver Metastases

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A phase Ib trial of a novel regional approach to adoptive immunotherapy is reported. Patients with liver metastases received continuous high-dose infusion of interleukin-2 (IL-2) into the splenic artery or intravenous infusion with subsequent transfer of lymphokine-activated killer (LAK) cells into the portal vein or the hepatic artery. Trafficking studies revealed homogenous distribution of the LAK cells within the liver. The usual side-effects of IL-2 and LAK cells occurred without limiting liver toxicity. One partial (7+ months) and two complete responses (36 and 26+ months) were observed in 9 patients with metastases from cutaneous melanoma. None of 6 patients with metastases from ocular melanoma responded.
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INTRODUCTION

ADOPTIVE IMMUNOTHERAPY with interleukin-2 (IL-2) and lymphokine-activated killer cells (LAK cells) has been used to treat human malignancies [1–5]. The role of *ex vivo* activated LAK cells in this type of treatment is controversial [6]. One possible reason limiting the efficacy of intravenously transfused LAK cells is the poor accessibility of tumour tissue outside the lung [7]. In animals, significant entrapment of LAK cells in tumour tissue has been achieved by regional arterial infusion [8, 9]. We, therefore, developed a novel regional approach of adoptive immunotherapy.

PATIENTS, MATERIALS AND METHODS

15 patients with unresectable progressive liver metastases of melanoma were studied (Table 1). 3 patients had failed chemotherapy and 4 immunotherapy with IL-2. Informed consent was obtained from all patients. The study was approved by the University of Heidelberg ethics committee.

Treatment protocols (Table 2)

In 8 patients, permanent catheters were inserted into the splenic artery and the portal vein. After continuous arterial infusion of natural IL-2 (nIL-2) (kindly provided by the German Red Cross, Springe) into the spleen, mononuclear cells were harvested from the peripheral blood for generation of LAK cells as described previously [10]. The LAK cells were transfused back into the portal vein, and the continuous perfusion of the spleen with nIL-2 was reinstituted. The subsequent 7 patients received recombinant IL-2 (rIL-2) (Eurocetus, Frankfurt, Germany) intravenously and the LAK cells were transfused into the hepatic artery. Access was obtained by an angiography catheter, temporarily placed into the hepatic artery via the femoral artery. Standard WHO response criteria were used. Treatment courses were repeated at 3-monthly intervals in the absence of disease progression.

In 4 patients (2 each with portal vein catheters and with hepatic artery catheters), 3×10^9 LAK cells were radiolabelled with ^{111}indium-oxine as described by Read [11] to monitor the *in vivo* distribution. IL-2 concentrations were measured by ELISA (Biermann, Bad Nauheim, Germany).

RESULTS

There was no difference observed in cell trafficking according to the route of administration (portal vein versus hepatic artery). More than 80% of transferred LAK cells were detectable in the liver immediately after transfer and after 24 and 120 h. The cell distribution within the liver was homogenous (Fig. 1). Most of the remaining radioactivity (10–15% at 120 h) was detected in the spleen. There was no significant systemic recirculation.

In 4 patients, the IL-2 concentration was determined in several simultaneously drawn samples from the portal vein and a central line. No difference in the concentration was observed between the portal vein and the peripheral blood at any point in time (data not shown).

The transfer of LAK cells was well tolerated by most patients. 5 patients developed right upper abdominal pain lasting for 30–60 min. Liver toxicity did not differ from that observed during systemic adoptive immunotherapy. The median peak value and range for serum glutamic-oxalacetic transaminase (sGOT) was 56 U/ml (34–98), 398 U/ml (198–897) for alkaline phosphatase, and 2.1 mg/dl (1.1–5.6) for bilirubin. The systemic side-effects with IL-2 and LAK cell therapy (flu-like syndrome, fluid retention and moderate hypotension) did not obviously

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Table 1. Patients' characteristics

Patient number	Site of primary	No. of liver lesions	Extrahepatic metastases	Pretreatment	Time pretreatment to regional immunotherapy	Response to treatment
1	Eye	>10	No	Chemotherapy	3 months	PD
2	Skin	>10	Spleen	Chemotherapy	2 months	PD
3	Eye	>10	No	Chemotherapy	2 months	PU
4	Skin	7	Skin	None		CR
5	Skin	2	No	None		SD
6	Eye	>10	No	None		PD
7	Skin	2	Skin	None		CR
8	Skin	7	Skin	None		PD
9	Eye	>10	No	None		PD
10	Eye	>10	No	None		PD
11	Eye	>10	No	None		PD
12	Skin	5	No	None		SD
13	Skin	1	No	None		PR
14	Skin	3	No	High-dose IL-2	2 months	PD
15	Skin	>10	Duodenal mucosa	Low-dose IL-2	2 months	PD

Table 2. Treatment schedules

Day	Schedule 1 (patients 1–8) nIL-2 (BRMP-U/m²/24 h)	Schedule 2 (patients 9–15) rIL-2 (CU/m²/24 h)
1	3×10^6	3×10^6
2	3×10^6	3×10^6
3	1.5×10^6	1.5×10^6
4	1.5×10^6	1.5×10^6
5		
6	Leukapheresis I	Leukapheresis I
7	Leukapheresis II	Leukapheresis II
8	Leukapheresis III	Leukapheresis III
9	Leukapheresis IV	Leukapheresis IV
10	3×10^6 <-LAK I + II	4.5×10^6 <-LAK I – IV
11	3×10^6	3.0×10^6
12	1.5×10^6 <-LAK III + IV	1.5×10^6
13	1.5×10^6	0.25×10^6
14	1.5×10^6	0.25×10^6

BRMP-U, biological response modifiers project units. CU, cetus units.

differ from intravenous administration. In 3 patients, the IL-2 dose was reduced to 50% on day 11 because of exhaustion and hypotension. In another patient with a history of gastric ulcers, treatment was discontinued on day 11 because of gastrointestinal haemorrhage. Two catheter infections and one leakage were observed. No patient receiving LAK cells via transient arterial catheterisation developed catheter-related complications.

Objective responses were observed in 3 patients with metastases of cutaneous melanoma: 2 complete response (CR) for 24+ and 36 months, and 1 partial response (PR) for 9+ months. Two additional patients had stable disease (SD) (10 and 11 months). In the patient with PR the lesion became surgically resectable. Histology revealed almost complete necrosis of the tumour tissue and a surrounding capsule of lamellar fibrosis with a small rim of lymphocyte infiltration between the capsule and the normal liver. The absence of any tumour response in all 6 patients with metastases from ocular melanoma is striking.

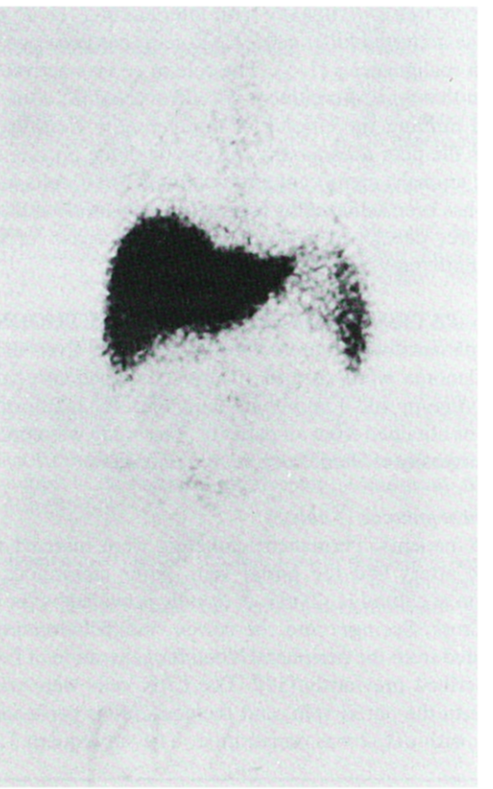

Fig. 1. Typical example of the *in vivo* distribution of LAK cells. 3×10^9 cells were radiolabelled with 250 μ Ci of ^{111}indium-oxine and injected into the portal venous catheter. The *in vivo* distribution was monitored at 0, 24 and 120 h postinfusion. The figure shows the total body scan after 24 h. At this time, 86% of radioactivity was detectable in the liver and 12% in the spleen.

In 3 of the responding patients, who presented with small extrahepatic lesions, a simultaneous regression of the extrahepatic metastases occurred. In patient 4, regression of the liver metastases was not seen following one systemic treatment cycle with IL-2 and LAK cells, but repeated regional infusion of LAK cells resulted in a complete remission, as described in detail previously [10].

In patient 7 with CR of liver metastases, brain lesions appeared after the second treatment cycle. Following successful irradiation of the brain metastases, no relapse in the liver has been observed after 29 months follow-up.

DISCUSSION

Our main objective was to administer as many LAK cells to the tumour tissue as possible. Indeed, after perfusion of the liver, more than 85% of the transferred LAK cells remained in the liver. The number of LAK cells in relation to the mass of perfused tissue was in the same order of magnitude as the number of cells used in experimental mouse models for systemic treatment [12, 13]. Perfusion of the liver with large numbers of LAK cells was feasible without limiting liver toxicity.

In 8 patients, IL-2 was also administered regionally, but the IL-2 levels in the portal venous blood were equal to the systemic circulation, and also similar to serum concentrations following systemic administration [14]. In accordance, no advantage of arterial perfusion of the spleen with IL-2 over systemic administration has been reported [15]. These observations suggest that the biodistribution of continuously infused IL-2 in the vascular system is similar during regional and systemic intravascular application, without evidence for a significant first pass effect in spleen or liver.

To our knowledge, there are no studies comparing cell distribution in liver metastases following portal venous or hepatic arterial administration. An advantage of the portal route is the lack of anatomical variations, but laparotomy is necessary for the placement of permanent catheters. Transient arterial catheterisation reduces the risk of catheter-related complications. We observed tumour regression using the portal vein as well as the hepatic artery.

Patients with unresectable liver metastases of cutaneous melanoma have a median survival of 4–6 months. We observed three durable objective remissions and two SD for almost a year. These results demonstrate the activity of the treatment regimen, although the number of patients is too small for comparison with other modalities. In 4 patients with cutaneous melanoma, no tumour regression was observed. In 1, the immunotherapy was preceeded by aggressive polychemotherapy, which may have decreased immunological responsiveness. 2 other patients had previously failed systemic high-dose or low-dose IL-2 treatment. In both cases, IL-2 antibodies (IgG) were detectable in the serum prior to regional treatment, which may have impaired the effects of IL-2.

No extrahepatic disease progression was observed in any patient with responding liver lesions with the exception of the development of brain metastases in 1 patient. Relapses in the brain following successful immunotherapy with IL-2 have also been observed by others [16], suggesting that the accessibility of the central nervous system for immunologic effector cells is limited by the blood–brain barrier.

The lack of response in the 6 patients with liver metastases from ocular melanoma is in accordance with poor results observed with systemic cytokine treatment [17], and with the differences in tumour biology between cutaneous and ocular melanoma [18].

The importance of regional cell transfer is especially supported by the course of patient 4 [10]. In this patient with an anatomical variation of hepatic blood supply, tumour regression after consecutive treatment cycles was only observed in anatomical areas of the liver which were perfused with LAK cells.

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