Enhancement of leukocyte adhesion after percutaneous irradiation in rats with hepatocellular carcinoma

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AIM: To evaluate the effects of percutaneous radiation on leukocyte-endothelium interaction (LEI) in experimental hepatocellular carcinoma (HCC).

METHODS: Twelve ACI rats underwent HCC-inoculation, six of which on day 12 received low-dose external radiation and six did not. After 12 h intravital microscopy was performed.

RESULTS: LEI was significantly reduced in tumor tissue. However, irradiation of liver sinusoids and tumor tissue with 6 Gy led to a significant activation of leukocyte adhesion in the tumor with a marked increase of the proinflammatory cytokine TNF-α.

CONCLUSION: The findings indicate that the immunological tumor-endothelial barrier can be overcome by external irradiation.

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Key words: Radiation; Hepatocellular carcinoma; Immune response; Animal model

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INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) is increasing worldwide, and represents the third most common cause of cancer-related death[1]. Although complete surgical resection is the primary goal, long-term survival is often limited by local recurrence or distant metastases of the tumor.
hepatoma A3294 was performed in 12 ACI rats. The recipient rats were anesthetized and the left liver lobe via a small midline incision was prepared for tumor implantation. Five million Morris hepatoma tumor cells (5 µL) were injected in subcapsular position. On d 12, six animals underwent a percutaneous single dose radiation with 6 Gy under general anesthesia. After 12 h the animals underwent laparotomy and intravital fluorescence microscopy was performed to determine tumor vessel diameter, red blood cell velocity (RBV) and leukocyte adherence. Values were compared to control animals without irradiation.

All animals were killed at the end of videomicroscopy and the whole liver was harvested for histopathological investigations. Serum specimens were taken at the end of experiments for enzyme analysis and TNF-α values.

Videomicroscopy
Intravital videomicroscopy was used according to the epiillumination technique reported by Menger et al. In general anesthesia the left liver lobe was exteriorized after relaparotomy. A Leitz fluorescence microscope (Leitz GmbH, Wetzlar, Germany) was used. In the presence of different excitation filters (wavelength 450-490 and 530-560 nm) visualization of FITC-labeled erythrocytes (Fluorescein isothiocyanate Isomer 1, Sigma, St. Louis, USA) and leukocytes with rhodamine 6G (0.02 mg/kg body-weight, Sigma) was possible. For contrast enhancement of plasma, FITC-labeled albumin was administered intravenously during experiments (50 mg/kg body-weight, Sigma). The microscopy was videotaped and off-line analysis was performed using a computer-assisted processing system.

The following parameters were assessed in ten randomly selected tumor areas and fields of healthy liver tissue. RBV was measured using the frame to frame method offline. Volumetric blood flow (V_b) was visualized after intravenous injection of FITC-labeled erythrocytes and analyzed offline. Determinants were erythrocyte velocity and vessel diameter (D) using the following equation: V_b = 4π/3 × V_c × π × D^3/2. LEI described the flow behavior of white blood cells and differentiated between low-affinity leukocytes (roller) moving with less than 66% of RBV or adhering for less than 30 s to the endothelium and high-affinity leukocytes (sticker) adhering for more than 30 s to the endothelium surface.

Hemodynamics
Blood gas analysis and monitoring of heart rate and mean arterial blood pressure were performed via the cannulated left carotid artery at the beginning of experiments, 30 min after the onset of videomicroscopy and 2 h after microscopy (ABL 5, Radiometer GmbH, Willich, Germany).

Cytokine measurement (TNF-α)
On d 13, blood samples of radiated animals and controls were taken after videomicroscopy for TNF-α measurement using a standardized ELISA kit (Pharmingen, USA).

Histology
One part of the harvested liver was fixed in buffered formalin and prepared for staining with hematoxilin and eosin to confirm tumor presence.

Statistical analysis
The data were expressed as mean±SD and compared between groups by Wilcoxon-Mann-Whitney U-test. P<0.05 was considered statistically significant.

RESULTS
There were no significant differences between the study groups in mean arterial blood pressure and blood gas analysis during intravital microscopy.

Control hemodynamics and blood gases were maintained at physiological levels throughout the experiments. Vessel diameter and basal RBV were comparable in hepatic tumor tissue and healthy liver tissue (Tables 1 and 2). There was a homogenous but not significant increase in volumetric blood flow in both groups (Table 3).

The number of high-affinity leukocytes was comparable in tumor tissue and healthy liver tissue (P>0.05) (Figure 1). After percutaneous low-dose irradiation (6 Gy), high-affinity LEI was significantly enhanced in tumor tissue and sinusoids (P<0.05) (Figure 1). TNF-α levels were significantly elevated after radiation (P<0.05) (Figure 2).

DISCUSSION
The results of the current study indicate that LEI decreases significantly in tumor tissue under basal conditions, but this can be overcome by low-dose external radiation. We have previously shown that tumor-associated endothelial cells have a suppressed expression of ICAM-1 compared to endothelial cells from healthy liver. Thus, only the basal expression of endothelial adhesion molecules is decreased in the tumor vasculature, but the possibility of inflammation-mediated upregulation is not hampered.

| Table 1 | Red blood cell velocity (RBV) (mm/s, mean±SD) |
|---------|---------------------------------------------|
|         | Healthy liver                              | Liver cancer                          |
| Controls | 1.49±0.3                                   | 1.85±0.12                             |
| Radiation| 1.88±0.1                                   | 1.93±0.14                             |

| Table 2 | Vessel diameter in liver tumor tissue and healthy hepatic parenchyma (µm, mean±SD) |
|---------|----------------------------------------------------------------------------------|
|         | Healthy liver                              | Liver cancer                          |
| Controls | 34.5±3.8                                  | 36.0±3.71                             |
| Radiation| 35.6±2.82                                 | 36.5±0.92                             |

| Table 3 | Volumetric blood flow pattern in liver tumor tissue and healthy liver parenchyma (nL/s, mean±SD) |
|---------|---------------------------------------------------------------------------------------------------|
|         | Healthy liver                              | Liver cancer                          |
| Controls | 0.25±0.07                                  | 0.29±0.02                             |
| Radiation| 0.31±0.03                                  | 0.35±0.05                             |
liver sinusoids and tumor vessels after radiation indicates that endothelial cells can be activated probably by unspecific radiation-induced inflammation with a marked increase of proinflammatory cytokine TNF-α.

Radiotherapy with or without transarterial embolization and/or percutaneous ethanol injection appears effective in controlling HCC and can prolong survival[17] although this is still controversial. External beam radiation is rarely used because TACE has a limited effect on portal vein tumor thrombus and pericapsular invasion of the tumor. This approach may provide response rates of 50% of treated infections during the revascularization of regenerating rat liver. Hepatology 2001; 34: 1135-1148

If tumor tissues are associated with tumor infiltrating lymphocytes at a high density or with sinus histiocytosis in its regional lymph nodes, good postoperative survival rates for cancer have been reported[2,21]. Involvement of an anti-tumor effect via cellular immunity, humoral immunity or via cytokines produced by the cancer cells has been discussed. TNF-α plays a critical role in the immune defense against tumor growth. By a regional infusion of the cytokine TNF-α and interferon-γ a significant reduction in tumor growth can be described in an animal model[23].

We focused on microcirculatory parameters and the course of TNF-α after radiation. Quantification of the LEI and determination of the proinflammatory cytokine TNF-α showed a significant increase after external single-dose radiation. These findings are in line with those of earlier studies, indicating that inflammation-mediated upregulation of adhesion molecules in tumor endothelium is possible[8,20].

Angiogenetic factors are capable of inducing a state of endothelial cell anergy. After activation by inflammatory cytokines there is a suppressed response of tumor endothelial cells compared to endothelial cells from normal tissue in human umbilical vein and human renal cell carcinoma[27]. This state is induced at the protein level (expression) and at the functional level (adhesion) and may serve as a tumor-

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