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Interactions between malignant tumor growth and allogeneic graft rejection in an experimental rat model

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Abstract We describe a combined tumor and simultaneous transplant model in rats in tended to investigate interactions between tumor growth and graft rejection. To study the influence of tumor growth on graft rejection. Novikoff hepatoma cells were injected subcutaneously into the back of Lewis rats. Eight days later, the grown solid tumor was resected, and allogeneic heart transplantation was performed. Four groups were formed, receiving 5-fluorouracil (5-FU), cyclosporin A (CsA), 5-FU + CsA, and placebo, respectively. In the corresponding groups, tumor injection was omitted. Graft survival was significantly prolonged when CsA was given 5-FU did not abrogate or augment CsA efficiency nor influence graft survival when given alone. In the corresponding control groups, graft survival was similar, thus excluding an immunomodulating effect of the prior tumor growth on graft survival. To study the reverse interaction of allogeneic graft on tumor growth, heart grafting and tumor cell injection were performed on the same day. In different groups, 5-FU, CsA, 5-FU + CsA, and placebo was given. For the control, no transplantation was carried out. The tumor was resected on the 8th postoperative day and examined by immunohistology. A slight decrease of tumor growth by 5-FU, but a marked increase by CsA were found, whereas the graft alone showed no immunomodulation.

Key words Tumor growth · Graft rejection · Heart transplant, rat

Introduction

The indications for liver transplantation because of malignancy are becoming more restricted because of the discouragingly high rate of local tumor recurrence and of distant metastases. However, the interactions between tumor growth and graft rejection, above all with regard to immunosuppressive and cytostatic therapy, have not been well explored. To study these interactions, we searched for an experimental model which combines a tumor model and a transplant model.

Materials and methods

For the transplantation model [8], we used an allogeneic heart transplantation (LEW × BN – LEW). Graft function was assessed by daily palpation. Rejection was taken as the complete cessation of

myocardial activity. The Novikoff hepatoma [6], a malignant hepatic tumor induced by feeding 4-dimethylaminoazobenzene, served as the tumor model: after subcutaneous injection of 5 times $\times 10^6$ cells into the rats back, a solid tumor arises.

In a first step, the influence of tumor growth on graft rejection was tested. In four groups ($n=6$), hepatoma cells were applied 10 days before, 3 days before, on the same day of, and 3 days after allogeneic heart grafting. The heart beat was monitored, and the time of graft rejection was marked.

In a second step, we studied graft rejection after resection of a prior tumor. Again, hepatoma cells were injected. Eight days later, the subsequent solid tumor was resected. Immediately afterwards, allogeneic heart transplantation was performed. Four groups ($n = 6$) were formed, receiving 50 mg 5-FU/kg, 1.5 mg CsA/kg, 50 mg 5-FU + 1.5 mg CsA/kg, or placebo. In the corresponding control groups, only tumor cell application was omitted.

In a third step, the reverse interaction of impact of an allogeneic graft on tumor growth was studied. Heart grafting and tumor cell injection were performed on the same day. In different groups ($n = 6$), 5-FU (50 mg/kg), CsA (15 mg/kg), 5-FU + CsA, or placebo was applied. For the control, no transplantation was carried out. The tumor growth curve was measured. On the eight postoperative day, the tumor was resected and examined by histology (H&E), DNA analysis, and immunohistology.

Graft survival was calculated by the Kaplan-Meier method. Comparison of survival rates and tumor growth curves was made by using the log rank test [5].

Results

In the presence of malignant tumor, graft survival is prolonged. Without tumor cell application, Lewis recipients reject the histoincompatible Lewis × Brown-Norway hearts 7 days after transplantation. Tumor cell injection 10 days before transplantation extended graft survival significantly ($P = 0.019$) to 11.8 days. Tumor cell application 3 days before heart grafting prolonged graft survival, but not significantly, to 8.5 days ($P = 0.112$), on the day of transplantation to 10.6 days ($P = 0.053$), and 3 days after transplantation to 9.5 days ($P = 0.291$).

After resection of a prior solid only for tumor, graft survival was significantly prolonged in comparison with the placebo group only for CsA (17.7 days, $P = 0.011$). The cytostatic 5-FU did not abrogate or augment CsA efficiency (17.8 days, $P = 0.011$), nor influence graft survival when given alone (6.8 days, $P = 0.071$). For the placebo, graft survival was 8.3 days. In the corresponding groups, graft survival was similar (CsA 16.8 days, $P = 0.019$; CsA + 5-FU 22.1 days, $P = 0.019$; 5-FU = 9.3 days, $P = 0.143$; placebo 7.2 days).

In the third step, when we studied the reverse interaction of allogeneic graft on tumor growth, a marked increase of tumor growth after CsA was found (tumor volume 6.24 cm^3, $P = 0.0322$) in comparison with the nontreated group (3.34 cm^3). 5-FU could diminish CsA efficacy (4.13 cm^3, $P = 0.237$). The administration of 5-FU was followed by a slight decrease of tumor growth (2.23 cm^3, $P = 0.528$). In the corresponding group, when tumor growth was examined without a simultaneous allogeneic graft, the tumor volume was nearly identical (placebo 3.34 cm^3; CsA 6.37 cm^3; $P = 0.0321$; 5-FU 3.16 cm^3, $P = 0.745$; 5-FU + CsA 3.72 cm^3, $P = 0.532$).

H & E-stained sections of the resected tumors showed a vital solid tumor only if placebo was administered. If 5-FU was added, regressive alterations could be found. CsA application led to a large central tumor necrosis. In tumors which were treated with CsA and 5-FU, both central necrosis and regressive alterations were seen. No difference could be found between the grafted and the control group. Immunohistological examinations showed markedly reduced expression of T cells. Natural killer cells, and macrophages in the CsA-treated tumors.

Discussion

The background to our investigation was the unsatisfactory situation in the treatment of primary-hepatic cancer. Only about 20%–40% of these tumors can be treated by conventional hepatic resection; most liver malignancies are unresectable. Therefore, total hepatectomy and liver replacement have been regarded as a logical extension of partial hepatectomy [10]. However, the long-term results of liver transplantation carried out for malignant liver tumors are discouraging because of the high rate of local tumor recurrence and distant metastases [3, 7, 9, 11]. Therefore, the number of liver transplantations performed for malignancy has decreased continuously, down to a share of only about 15%, whereas the total number of liver transplants has increased over the same period [1]. In the future, transplantation will regain importance in the surgical treatment of hepatocellular carcinoma: New data suggest that the best indications are small tumors, which were, until now, generally considered for resection. In the treatment of these small tumors, better results can be achieved by allogeneic transplantation in comparison with conventional resection [2].

A precise understanding of the interactions, between tumor growth and graft rejection, above all with regard to immunosuppressive and/or adjuvant cytostatic therapy, is necessary. However, presently only very few studies exist which investigate these complex immunological interactions. In the literature, we could not find a generally applicable and suitable experimental model.

In our study, we introduced a new model which combines a transplantation model and a tumor model in

rats. For the transplantation model we performed heterotopic heart grafting [8] with the Lewis × Brown-Norway rat as the donor and the Lewis rat as the recepient. Heart grafting instead of allogeneic liver grafting was used, on the one hand because it is easy to conduct and on the other because the cessation of heart beat is a precise indicator of graft rejection. The Novikoff hepatoma [6] served as the tumor model because its morphology and biological behavior are very close to hepatocellular carcinoma [4].

In a first step, we tested the influence of a concurrent tumor on graft rejection. In a second step, graft rejection was studied after resection of a prior tumor. Finally, the reverse interaction of the impact of an allogeneic graft on tumor growth was studied. Our data revealed that graft survival was prolonged in the presence of a malignant tumor. However, graft rejection was not influenced by a prior tumor which was resected before transplantation. On the other hand, an influence of the allogeneic graft on tumor growth was not detectable. CsA prolonged graft survival and augmented tumor growth; 5-FU only had a marginal influence on graft rejection but could reverse the effect of CsA on tumor growth.

We think that our combined transplant/tumor model is suitable to investigate the interactions between graft rejection and tumor growth and justifies further studies.

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