EVALUATION ON FACTORS INFLUENCING LIVER CANCER METASTASIS AFTER LIVER SURGERY BY A MOUSE MODEL

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ABSTRACT

Objective: To evaluate the influence of surgical trauma on liver cancer metastasis. Methods: A mouse model of experimental liver cancer metastasis was established by subcapsule injecting hepatoma ascites tumor cells (H_{22}) into spleen of NIH mice. Simple intrasplenic inoculation, with sham operation, partial hepatectomy, total occlusion of hepatic blood inflow and blood loss and re-perfusion were performed and metastatic effects were observed. Results: There were significant higher metastasis-augmenting effects in sham operation and partial hepatectomy groups. Compared with no-blood transfusion, blood transfusion group was found to be potent to increase intrahepatic metastases. But, neither inhibition nor enhancement with total occlusion of hepatic blood inflow for 20 and 30 minutes was seen. Conclusions: Surgical trauma, especially partial hepatectomy and blood transfusion, are involved in enhancing metastasis, but total occlusion of hepatic blood inflow is not responsible for enhanced liver metastasis in the experimental metastasis model.

Key words: Primary hepatocellular carcinoma, Liver metastasis, Disease model, Hepatectomy, Surgical trauma, Blood transfusion, Mice

Intrahepatic recurrence is usually the reason for surgical failure after resection of primary hepatocellular carcinoma. In this study, we evaluated the influence of surgery trauma, total occlusion of hepatic blood flow and blood loss and re-perfusion on experimental liver cancer metastasis by establishing an experimental liver metastasis model in mice.

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MATERIALS AND METHODS

Animal Model and Groups

Female NIH mice, weighing 18-20 g, were obtained from the Animal Center of the PLA General Hospital. High metastatic hepatoma ascites tumor cells, H_{22}, were regularly passages on time every two weeks in NIH mice. Simple intrasplenic inoculation group: Anesthesia was achieved by intraperitoneal injection of sodium pentobarbital at 75 mg/kg. Ten microlitre H_{22} ascites tumor cells (1×10^6 tumor cells) were injected into the spleens of the mice through a 0.5 cm incision at left middle abdominal wall. The mice were sacrificed by cervical dislocation and the liver were removed on days 9—11. The number of metastases on the surface of the liver was visually counted. Sham operation group: The mice underwent laparotomy, and their bowels were drawn extraperitoneally and then covered by gauze wetted with saline. After various lengths of time, the gauze was removed and the wound was closed. Partial hepatectomy group: The liver tissue of the mice weighing 200 and 300 mg respectively, about 1/4 and 1/3 of total liver volume, were resected; other steps were similar to sham operation. Total occlusion of hepatic blood inflow goup: Hepatic pedicle was occluded for 20 and 30 minutes at porta hepatis, other steps were similar to sham operation. Blood loss and re-perfusion group: Homologous blood, stored at 4℃ for 4 days, was transfused intraperitoneally immediately into the mice after partial hepatectomy. All of the latter four groups were given intrasplenic inoculation.

Stage of Metastases on Liver Surface

Table 1. Revised staging criteria of liver metastatic cancer in mice model[1]

Stage	Metastasis Presentation
0	No metastasis
I	1 — 2 metastases those are 0.2 — 0.5 mm in diameter, one lobe involvement only
II	3 — 5 metastases, those are 0.2 — 0.5 mm in diameter, or 1—2 metastases, 0.5—1 mm in diameter
III	Multiple metastases larger than 1mm but less than 1 cm in diameter, no total liver involvement
IV	Multiple metastases more than 1 cm in diameter or total liver involvement

Analytical Methods

The probability of significant differences between the above groups were determined by Ridit's analysis.

RESULTS

Influence of Surgical Trauma on Liver Metastasis

The mice underwent sham operation and partial hepatectomy in order to analyze whether surgery-bearing mice showed an enhanced liver metastasis. The results suggested that the mice with sham operation and partial hepatectomy have enhanced liver metastatic effects compared with control mice (single intrasplenic inoculation) ($P<0.001$). There is also a significant difference between the two groups with surgery ($P<0.05$), more enhanced effects of liver metastasis in partial hepatectomy group.

Time Effects of Partial Hepatectomy on Liver Metastasis

To investigate the time course of surgery-induced augmentation of liver metastasis, mice were given intrasplenic inoculation at various times before or after partial hepatectomy. The results show that similar enhanced effects of liver metastasis were only observed in the mice with intrasplenic inoculation given 2 and 3 days before partial hepatectomy compared with control mice (partial hepatectomy and intrasplenic inoculation given 5 days before or 3 and 7 day after partial hepatectomy).

Volume Effects of Partial Hepatectomy on Liver Metastasis

The mice underwent 200 and 300 mg volume of liver tissue resection. The result shows that the mice with 300 mg volume resected have higher grade to metastasis to metastasize compared to the mice with 200 mg volume respectively. ($P<0.05$)

Influence of Total Occlusion of Hepatic Blood Flow on Liver Metastasis

A comparison of metastasis between mice with and without total occlusion of hepatic blood flow for 20 and 30 minutes was analyzed. The result shows that no significant difference was detected.

Influence of Blood Loss and Re-perfusion on Liver Metastasis

The mice were given immediately homologous blood transfusion after partial hepatectomy. The result shows that there is an enhanced liver metastasis effect in the mice with blood transfusion compared to the mice without blood transfusion ($P<0.001$).

DISCUSSION

Many surgeons have the impression that more extensive surgery associated with a greater blood loss and logically a greater inflammatory response seems to increase local and distant recurrence rate, not just for liver cancer but also for most malignant solid tumors.[2-4] Removal of liver tissue, hepatic blood flow occlusion and massive blood transfusion are common during hepatic surgery; for these reasons, mechanisms of surgical trauma involved in liver metastasis-enhancing effects were investigated by an experimental mouse model that was established by intrasplenic inoculation of H_{22} ascites tumor cells. In this experiment, the mice with sham operation and especially partial hepatectomy exhibited higher liver metastatic activity than mice with simple intrasplenic inoculation. A significantly enhanced liver metastatic effect was seen in the mice with 1/3 hepatectomy compared to those of 1/4 hepatectomy. The results indicated that the degree of surgical trauma correlated with liver metastasis promoting effect. A comparison between groups of partial hepatectomy at various periods of intrasplenic inoculation given to mice was analyzed in order to demonstrate further the influence of time course of surgical trauma on liver metastasis-augmentig effects. The mice with intrasplenic inoculation given 2 or 3 days prior to partial hepatectomy showed significant liver metastasis-enhancing effects similar to those mice with partial hepatectomy and intrasplenic inoculation given at the same time, whereas in the mice inoculation given 5 days prior to partial hepatectomy no such enhanced effect was demonstrated. In contrast, the mice that had partial hepatectomy 3 or 7 days prior to intrasplenic inoculation showed no detectable liver metastasis-

enhancing effects compared to the mice with partial hepatectomy and intrasplenic inoculation given at the same time. That is to say, the enhanced liver metastasis caused by partial hepatectomy was found most strikingly when the tumor cells were inoculated within 3 days before or after the surgery, but was no longer observed over 3 days. This suggests that the transient nature of the mechanism is responsible for the surgical-trauma-enhanced metastasis. Pathological examination showed that, for first three days after inoculation, the surface of the liver could be seen studded with multiple white ischemic necrosis areas, these were due to occlusion of small arterioles stopped with tumor cells, which could be detected by microphotoscopy and there was no significant difference between the two groups. Besides, mice with partial hepatectomy also showed vacuolar degeneration of the liver cells. After three days, white ischemic necrosis observed on the surfaces of the livers disappeared and tumor cells invaded the liver tissues and colonized with more remarkable progression in the partial hepatectomy group than the mice with simple intrasplenic inoculation. In this study, the metastasis-augmenting activity induced by surgical trauma, especially by partial hepatectomy, was demonstrated, but, this was not due to the increase of tumor cell burden to the remaining portion of the liver tissue since such effect was found in the animals when inoculation was given more than 3 days before or after partial hepatectomy. Blood transfusions given immediately after partial hepatectomy were seen to have metastasis enhancing effect in comparison with those without blood transfusion. Similar phenomena were found in patients with malignant solid tumors.[5-7] But, the molecular mechanisms of this phenomena have not been clarified. Total occlusion of hepatic blood inflow at porta hepatis gives rise to anoxia and ischemia of the liver. The ischemic effect may cause injury of the vascular endothelia of liver sinusoid. But no significant enhanced-metastasis effect was observed in the mice with occluded total hepatic blood inflow for 20 and 30 minutes in this experiment. Tumor metastasis is a complex process involving the release of tumor cells from a primary tumor site, the entering into the vascular or lymphatic circulation and attaching to the endothelium of vessels or capillaries before they can break through the vessel wall and inducing angiogenesis. Tumor cell adhesion to capillary endothelial cells is a critical step in metastatic processes. Animal experiment showed that, when i.v. administrate tumor cells for 24 hours, about 1% of which could be alive, 0.1% would invade and infiltrate into host target organs.[16] In fact, for patients with malignant solid tumors, tumor cells are constantly released from the primary site into vascular and lymphatic circulation, but only a part of the patients may be found with clinical metastasis. Manipulation during surgery may give rise to dislodgment of a large amount of tumor cells into circulation, which might partially explain the postoperative enhanced metastastic phenomenon. On the other hand, some other pathophysiological mechanism may be involved in liver metastasis-enhancing effects after surgical trauma. (1), In previous study, serum levels of acute response proteins stimulated and activised by surgical injury including TNF-a, IL-1 and IL-6 were found to be high in human and mice after surgery. The peak level of TNF-a was observed at 15 and 48 hours in human,[11] and 12 hours in mice,[12] IL-1 at 18 and 72 hours in human[11] after surgery. The previous report[9] demonstrated that i.v. administration of recombinant TNF-a enhanced the lung metastasis of B16-BL6 by up-regulating the expression of VCAM-1 on vascular endothelium which binds to VLA-4 on the tumor cell. IL-1 may play an important role in the events concomitant with liver invasion by blood-borne tumor cells.[10] Tumor cells transiently residing in the hepatic sinusoidal bed can take advantage of TNF-a and IL-1-mediated increase in adhesion to the capillary wall. TNF-a activity also result in phenotypic change of the endothelium characterized by an increased permeability and in the induction or upregulation of various cell adhesion. Therefore, it can be speculated that the transient enhanced metastatic effects in the first 3 days after surgery is related directly to the transient high levels of these acute response cytokines and various adhesion molecules induced by surgical trauma. The data collected in the current investigation also support the idea. (2), Patients undergoing hepatectomy for liver cancer also suffered from suppressed immune response. This includes low NK activity, low antibody-dependent cell-mediated cytotoxicity (ADCC) activity, and a decrease in the number of lymphocyte.[14,15] So, the post-traumatic immunosuppression can result in spread and progression of tumor cells.[8] (3), Liver regeneration takes place after partial hepatectomy and liver injury, and expression of HGF mRNA and HGF plasma level are increased. HGF is a mitogen for normal hepatocytes,[13] but the influence of HGF in liver regeneration on postoperative liver metastasis has not been clarified.

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