SPANDIDOS PUBLICATIONS

Increased growth factor expression after hepatic and pancreatic resection

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Abstract. Removal of the primary tumour is suggested to associate with an enhanced tumour growth of residual micro-metastases. Recent data focus on growth factors that may be released in response to surgery-stimulating receptors of residual tumour cells. Vascular endothelial (VEGF) and hepatocyte growth factor (HGF) are potent inducers of angiogenesis. The two factors are necessary for wound healing and the promotion of tumour growth. This study was designed to determine growth factor serum levels in patients before, during and after major abdominal surgery. It was recently shown that simultaneous hepatic and pancreatic resection led to poor liver regeneration. As growth factors may be involved in these findings we compared the growth factor levels after liver resection with the levels in patients after pancreatic resection. Forty patients were accrued before hepatopancreatic surgery (hepatic resection n=20 and pancreatic resection n=20). Blood samples were taken from each patient immediately prior to surgery, during the operation and on the postoperative days (POD) 1-3, 5 and 10. To examine the wound fluid, liquid from the wound drains was collected on POD 3. Using ELISA the concentration of the angiogenic cytokines HGF and $VEGF_{165}$ was determined. After the liver and pancreatic resections, circulating HGF and $VEGF_{165}$ were increased. We found significantly higher levels of HGF on POD 1-3 ($p<0.01$), compared to preoperative results with a peak on POD 2. After measuring the postoperative $VEGF_{165}$ levels we found significantly higher levels of circulating $VEGF_{165}$ on POD 1-5 ($p<0.01$) compared to the preoperative levels. On comparing liver with pancreatic resection we did not detect significantly different levels of the two growth factors in the two groups. $VEGF_{165}$ and HGF concentrations measured during the operation demonstrated no change. HGF and $VEGF_{165}$ levels detected in the wound fluid on POD 3 were ~10 times higher than the preoperative serum levels, respectively. In summary, our data show increased $VEGF_{165}$ and HGF levels after hepatopancreatic surgery. Notably, the lack of an impact of the type of organ resection on the concentration-time curve of the two growth factors suggest that high postoperative growth factor levels are part of normal wound healing and systemic inflammation. Thus, the proangiogenetic potential of growth factors may account for accelerated tumour growth when residual tumour cells are exposed to high levels of $VEGF_{165}$ and HGF.

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Introduction

Primary and metastatic hepatic and pancreatic tumours are a common cause of death worldwide. Surgical treatment is the only curative option available for patients. Previous studies suggest that removal of the primary tumour can be associated with an enhanced tumour growth of residual micro-metastases (1). Experimental animal data show that metastatic growth after liver resection is significantly accelerated in the course of hepatic regeneration (2-5). However, the exact mechanism of this enhanced tumour growth remains unclear. Allendorf *et al* showed that cell-mediated immune function is affected after open surgery leading to increased tumour growth (6). Some authors have suggested that surgical excision may remove a variety of growth factor inhibitors resulting in angiogenesis and the subsequent growth of previously dormant metastases (7,8). Recent data focus on growth factors released in response to surgery-stimulating receptors of residual tumour cells (9,10).

Hepatocyte growth factor (HGF) is a mesenchymal cytokine with a number of biological activities including mitogenic and morphogenic properties in a variety of epithelial tissues. HGF is also known as an angiogenic factor that promotes endothelial cell growth, survival and migration (11,12). It was first described in the blood of partially hepatectomized rats as a potent stimulator of growth in hepatocytes and neoplasms (13). The treatment of tumour cells *in vivo* with HGF results in an increase in the metastatic potential of the treated cells (14,15).

Vascular endothelial growth factor (VEGF) is a potent mediator of angiogenesis inducing new vessel formation, endothelial cell proliferation and migration (16). Moreover,

VEGF was shown to promote tumour growth (17). The release of VEGF and HGF after surgery may result in the promoted growth of residual tumour cells. Although increased plasma levels of growth factors were measured after gastric and colorectal surgery very few data report on the changes in serum and peritoneal fluid growth factor concentrations after hepatopancreatic surgery (9,10,18). The aim of this study was to the determine peri- and postoperative $VEGF_{165}$ and HGF levels in patients undergoing hepatic or pancreatic resection. Simultaneous hepatic and pancreatic resection leads to poor liver regeneration in animal models (19,20). As growth factors may be involved in these findings we compared the growth factor levels after liver resections with the levels in patients after pancreatic resection.

Materials and methods

Patients. The study was approved by the local ethics committee of Saarland, Germany. Informed consent was obtained from all patients before study inclusion. Forty patients were accrued before hepatopancreatic surgery (20 patients before hepatic and 20 patients before pancreatic resection) (Table I) between May 2006 and July 2007. Information regarding surgical indication, clinical and pathological staging (cancer patients), demographics, intra- and postoperative course, time in ICU and in hospital were prospectively collected for all study patients (Table II). One blood sample was taken from each patient immediately prior to surgery and two samples were taken during the operation (one immediately after laparotomy and one after liver or pancreatic resection). In addition, six blood samples were taken on the postoperative days (POD) (one sample 1 h after the operation and POD 1-3, 5 and 10). To examine the wound fluid, liquid from the wound drains was collected on POD 3.

Exclusion criteria. Patients with liver cirrhosis (>Child A), chronic inflammatory diseases and patients with chronic viral infections such as hepatitis and HIV were not included. We excluded one patient after surgical revision within 24 h after the first operation.

Blood sampling and processing. Peripheral blood samples and wound fluid were collected in heparin-coated tubes. After being drawn plasma was isolated from samples by centrifugation at 1500 g for 5 min and then stored at -80˚C until ELISA assays were performed.

$VEGF_{165}$ and HGF concentrations. All samples were assayed in duplicate using commercially available ELISA kits (Quantikine kit, R&D Systems, Abingdon, Oxon, UK) following the manufacturer's guidelines.

Statistical analysis. All statistical analyses were performed using the SPSS software (sigma stat 3.0).

Results

Characteristics of patients and perioperative growth factor levels. Prior to hepatopancreatic surgery, 40 patients were enrolled in this study over a 14-month period. The median age of patients was 64 (35-82 years). There were 25 male (62.5%) and 15 female patients. The demographic and operative data are shown in Tables I and II.

Table I. Type of operation.

Liver resection	20
Left hemihepatectomy	3
Right hemihepatectomy	2
Segmental resection (>1 segment)	15
Pancreatic resection	20
Pylorus-preserving pancreaticoduodenectomy	16
'Classic' pancreaticoduodenectomy	2
Left pancreatic resection	2

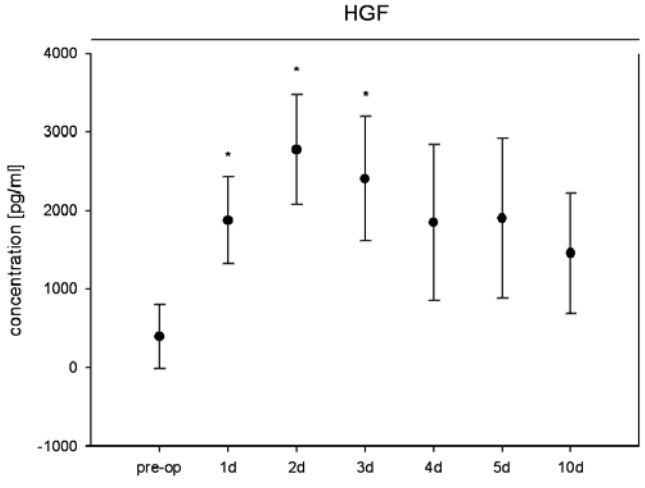

Figure 1. Concentration of serum HGF in patients undergoing hepatopancreatic surgery. Sampling points were preoperative (pre-op), and on postoperative days 1-5 and 10. *p<0.01 (compared with preoperative levels).

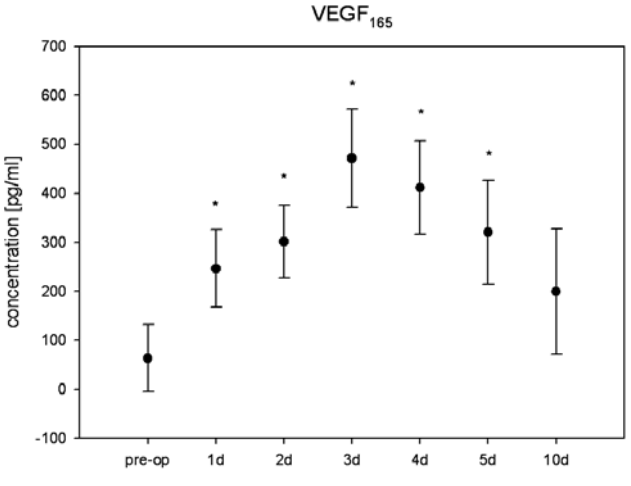

Figure 2. Concentration of serum VEGF in patients undergoing hepatopancreatic surgery. Sampling points were preoperative (pre-op), and on postoperative days 1-5 and 10. *p<0.01 (compared with preoperative levels).

Demographics and indication.

	Hepatic resection		Pancreatic resection	
N	20		20	
Age (years)	64.5 (35-82)		63.2 (45-75)	
BMI	24.72		24.4	
ASA	2.4		2.5	
Operation time (min)	202 (121-247)		216 (138-330)	
ICU (days)	3.4 (1-8)		3.8 (2-10)	
IHOS (days)	12.9 (8-23)		13.4 (7-28)	
Indication	Metastatic colon carcinoma	n=15	Carcinoma pancreas	n=11
	Metastatic mamma carcinoma	n=1	Carcinoma duodenal papilla	n=4
	HCC	n=3	Cystadenoma	n=2
	Seminoma	n=1	Endocrine tumour	n=2
			Adenoma duodenal papilla	n=1

BMI, Body mass index; ASA, Physical status classification system by the American Society of Anesthesiologists (1963); ICU, Patients stay in the intensive care unit; IHOS, In hospital stay; HCC, Hepatocellular carcinoma.

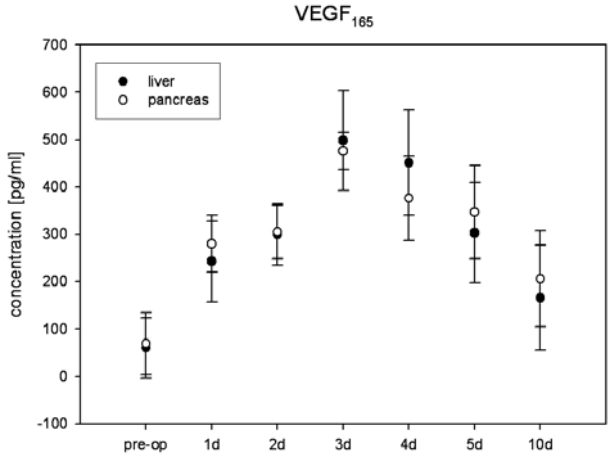

Figure 3. Concentration of serum VEGF in patients undergoing hepatic vs. pancreatic surgery. Sampling points were preoperative (pre-op), and on postoperative days 1-5 and 10. p>0.1 (comparing the liver with the pancreas group).

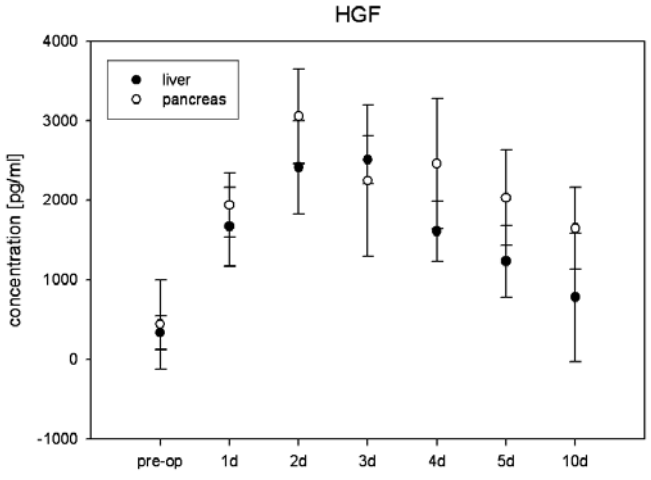

Figure 4. Concentration of serum HGF in patients undergoing hepatic vs. pancreatic surgery. Sampling points were preoperative (pre-op), and on postoperative days 1-5 and 10. p>0.1 (comparing the liver with the pancreas group).

The growth factor expression was evaluated in all patients before surgery. There was a great variation in the levels of the two growth factors before the operation (HGF: 394±424 pg/ml and $VEGF_{165}$: 63.4±68.3 pg/ml). Measuring $VEGF_{165}$ and HGF during the operation demonstrated no change in the growth factor concentrations (data not shown).

Postoperative levels of circulating factors. HGF and $VEGF_{165}$ serum levels were examined postoperatively (Figs. 1 and 2). After the liver- and pancreatic resection, circulating HGF was increased with a peak at 48 h. We found significantly higher levels of HGF on POD 1-3 compared to preoperative results ($p<0.01$; POD 1: 1878±951, POD 2: 2777±1237 and POD 3: 2406±1127 pg/ml). On POD 4, 5 and 10 we did not detect significantly higher plasma levels of HGF compared to the preoperative levels.

On measuring the postoperative $VEGF_{165}$ levels we found significantly higher levels of circulating $VEGF_{165}$ on POD 1-5 when compared with preoperative levels ($p<0.01$; POD 1: 246±79, POD 2: 301±74, POD 3: 471±107, POD 4: 411±138 and POD 5: 320±106 pg/ml). However, $VEGF_{165}$ peaked on POD 3 and nearly returned to preoperative levels on POD 10.

Growth factors after liver and pancreatic surgery. To determine whether pancreatic surgery had an impact on HGF and VEGF levels we compared the results of the 20 patients after liver surgery to the 20 pancreatic resections. The two groups were nearly similar with regard to age, gender, body mass index and operation time (Table II). We did not detect significantly different levels of the two growth factors in the two groups (Figs. 3 and 4).

HGF and $VEGF_{165}$ levels in peritoneal fluid. We measured the concentration of HGF and $VEGF_{165}$ in the wound fluid taken from the abdominal drains on POD 3. We found high levels of the two examined growth factors in the drain fluid (HGF: 4649±2140 pg/ml and $VEGF_{165:}$ 804±215 pg/ml). The detected HGF and $VEGF_{165}$ levels in the wound fluid on POD 3 were ~10 times higher than the preoperative serum HGF and $VEGF_{165}$ levels.

Discussion

Experimental data suggest that surgical trauma may be associated with increased tumour growth (21-24,31). Various potential mechanisms have been identified demonstrating a correlation between surgical trauma and tumour growth (6,25,26,31). However, these mechanisms were not linked to clinical outcome in cancer patients. Recent data focus on growth factors and angiogenesis and their influence on tumour growth. It is a well-known fact that normal wound healing is associated with angiogenesis and the release of proangiogenetic factors. This may be problematic for cancer patients as the proangiogenetic conditions may lead to accelerated tumour growth. This study aimed to elucidate whether surgical trauma may lead to elevated serum levels of growth factors.

Regardless of the surgical indication and organ resection, our studies demonstrated that major abdominal surgery was followed by a significant elevation of the two growth factors, VEGF and HGF, in serum and wound fluid. Few studies currently report on VEGF and HGF serum levels in the first 10 days after surgery.

VEGF plays a key role in wound healing (16). Therefore, serum VEGF levels may reflect the extent of wound healing after surgical traumas. The present study was the first to show there is no difference in $VEGF_{165}$ serum levels when comparing liver and pancreatic surgery. Other studies reported that elevated $VEGF_{165}$ levels after colorectal resections depended on the surgical method and incision length (9,27). These observations suggest that it is the surgical trauma rather than the disease that is associated with increased $VEGF_{165}$ levels.

HGF was the first growth factor described in the blood of partially hepatectomized rats and is a potent stimulator of growth in hepatocytes and neoplasms (13). There is increased synthesis of HGF by non-parenchymal cells after partial hepatectomy (28). In our investigation, we found increased HGF serum levels after liver and pancreatic resection. The serum HGF level changes in association with hepatocellular dysfunction, hepatic necrosis and systemic inflammation (29,30). Systemic inflammation as a response to major abdominal surgery may explain the elevation of HGF after hepatic and pancreatic resection.

In summary, our data suggest that wound healing and systemic inflammation response after surgical trauma lead to increased VEGF and HGF levels. This may explain the elevation of the examined growth factors only for the first days after the operation. In addition, we detected high levels of HGF and $VEGF_{165}$ in the drain fluid of our patients on POD 3. The observation that the growth-factor increase in drain fluid occurred regardless of the disease or type of surgical procedure suggests that it is a part of the normal early wound healing.

Since we did not find any impact of the type of operation and organ resection on the concentration-time curve of the two growth factors, our data do not explain the influence of pancreatic resection on liver regeneration.

Of note is what the elevated systemic levels of VEGF and HGF mean to cancer patients. It has been described that major surgery is associated with immunosuppression early after the surgical trauma (6). In this period no antiproliferative therapies such as chemotherapy are applied. However, the proangiogenetic potential of growth factors may account for accelerated tumour growth when residual tumour cells are exposed to high levels of VEGF and HGF. With a similar line of arguments Belizon *et al* see an alternative in antiangiogenetic therapy in the early postoperative period (9). It seems reasonable that elevated levels of proangiogenetic factors lead to accelerated tumour growth. In contrast, in the present study, major abdominal surgery was followed by significantly increased HGF and $VEGF_{165}$ levels only for the first days after the operation. Except for these findings scarce data exist on serum growth-factor levels in the first two weeks after the trauma. Whether accelerated tumour growth for a few days has an impact on patient prognosis has to be discussed critically. As postoperative wound healing and regeneration is a complex process it is inevitable that additional factors will be involved. Therefore, continuous research will lead to a better understanding of the early postoperative period and the effect of the surgical trauma on tumour growth.

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