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Intraperitoneal 5-Fluorouracil treatment of cancer – clinical and experimental studies

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…kill your darlings….

1. Contents 4
2. Abbreviations 6
3. List of publications 7
4. Introduction 8
5. Background 9
Pancreas cancer 9
Epidemiology 9
Clinical features 9
Surgical treatment 10
Adjuvant therapy 10
Palliative therapy 11
Intraperitoneal chemotherapy 12
Peritoneal blood flow measurements 15
Laser doppler flow 15
Inert gas clearance techniques 16
6. Aims 17
7. Materials and methods 18
Animals and preparation 18
Experimental tumours 18
Blood flow measurements 19
Intraperitoneal 5-Fluorouracil 22
Patients 22
Surgical procedure 24
Treatment schedule 25
Clinical evaluation 25
Pharmacokinetic study 25
Drugs 26
5-Fluorouracil 26
Leucovorin 27
Vasopressin 27
Statistical analysis 28
8. Results 29
Peritoneal blood flow 29
^{133}Xe-clearance technique 29
Laser doppler flow 31
Intraperitoneal 5-FU 32
Peritoneal carcinomatosis 33
Clinical studies 34
Feasibility of IP 5-FU treatment 34
Effect of IP 5-FU treatment 34
Safety of IP 5-FU treatment 35
Pharmacokinetic study 37

9. Discussion 38
Peritoneal blood flow 38
^{133}Xe-clearance technique 38
Intraperitoneal 5-FU 39
Peritoneal carcinomatosis 40
Influence of vasopressin 40
Clinical studies 41
Intraperitoneal chemotherapy 41
Feasibility 42
Safety 42
Tumour response 43
Selection of patients 44
Pharmacokinetics 45
Carrier solution 46
Tissue penetration 47
10. Considerations for the future 49
11. Conclusions 51
12. Popularised summary in Swedish 52
13. Acknowledgements 55
14. References 57

2. Abbreviations

AUC	Area under the curve
BSC	Best supportive care
Cmax	Peak concentration
CT	Computer tomography
DI	Dose intensity
DPD	Dihydropyrimidine dehydrogenase
EBRT	External beam radiotherapy
FBAL	Alfa-fluoro-ß-alanine
FdUMP	5-Fluoro-2´-deoxyuridine monophosphate
5-FU	5-Fluorouracil
FUTP	5-Fluorouridine triphosphate
Hep	3-Methyl-diaminobenzidine-induced syngeneic hepatoma
HPLC	High performance liquid chromatography
IORT	Intraoperative radiotherapy
IP	Intraperitoneal
IV	Intravenous
KI	Karnofsky index
LH	Lister-Hooded
LDF	Laser doppler flow
LV	Leucovorin
MAP	Mean arterial blood pressure
MDR	Multi drug resistance
mTHF	5,10-Methyl-tetra-hydrofolate
MRI	Magnetic resonance image
MTD	Maximal tolerated dose
NGW	N-methyl-N'-nitrosoguanidine-induced syngeneic adenocarcinoma
RECIST	Response evaluation criteria in solid tumours
PET	Positron emission tomography
TS	Thymidylate synthetase
UTP	Uridine triphosphate
VP	Vasopressin
W-Fu	Wistar-Furth
WHO	World health organisation
^{133}Xe	Xenon-133

3. List of publications

This thesis is based on the following papers, which will be referred to in the text by Roman numerals.

I. Öman M, Blind PJ, Naredi P, Gustavsson B, Hafström LO. Treatment of non-resectable pancreatic cancer with intraperitoneal 5-FU and leucovorin IV. Eur J Surg Oncol. 27(5):477-81, 2001.

II. Öman M, Tölli J, Blind PJ, Naredi P, Hafström LO. ^{133}Xe-clearance estimates the effect of vasopressin on peritoneal blood flow in rats. Hepatogastroenterology 51 (58):1037-41, 2004.

III. Öman M, Tölli J, Naredi P, Hafström LO. Effect of carcinomatosis and intraperitoneal 5-Fluorouracil on peritoneal blood flow modulated by vasopressin in the rat as measured with the ^{133}Xe-clearance technique. Cancer Chemother. Pharmacol. 54(3):213-8, 2004.

IV. Öman M, Lundqvist S, Gustavsson B, Hafström LO, Naredi P. Phase I/II trial of intraperitoneal 5-Fluorouracil with and without intravenous Vasopressin in non-resectable pancreas cancer. Submitted.

4. Introduction

Pancreas cancer is a most aggressive malignancy with dismal outcome for patients. The disease is usually advanced at presentation (*Haycox et al. 1998a*) and the aggressive biological phenotype is exceptionally resistant to all forms of therapy (*Magee et al. 2001*). Surgery offers the only possibility of cure, but even in those curatively resected, the median survival is short (13–18 months) and 5-year survival at best 15–26% (*Mosca et al. 1997; Kuhlman et al. 2004; Yeo et al. 1995*). Adjuvant chemotherapy sometimes supplemented by radiotherapy, aims to improve survival following curative resection by treating any residual microscopic disease. There is no convincing evidence of efficacy even if a recent randomised study indicated effect of IV 5-FU (*Neoptolemos et al. 2004*).

More than 80% of patients diagnosed with pancreas cancer are not suitable for resection due to the presence of locally advanced or macroscopically disseminated disease (*Warshaw et al. 1992*). These patients with unresectable pancreas cancer could be eligible for palliative treatment. In these patients, biliary decompression can be achieved operatively or by either endoscopic or percutaneous transhepatic techniques. The incapacitating tumour associated pain is most often relieved by morphine analgesics or less usual with surgical splanchnicectomy or chemical celiacectomy. Duodenal obstruction, incipient or manifest, can be mini-invasively treated with placement of duodenal stents or operatively by a gastroenteric anastomosis. The overall median survival from diagnosis for patients given best supportive care is 3–5 months with a 12-month survival rate of around 10% and a 5-year survival rate of 0.4–3% (*Bramhall et al. 1998*).

Systemic palliative chemotherapeutic treatment for pancreas cancer has low efficacy despite considerable toxicity, with only 2-3 months prolongation of survival (*Palmer et al. 1994; Permert et al. 2001; Scheithauer et al. 2003; Novarino et al. 2004*). The advantage compared to best supportive care, that motivates chemotherapy, is not prolonged survival but improved well-being (*Glimelius et al. 1996*). The importance of low-toxicity drugs must be stressed, as the limited gain in survival should not be at the cost of quality of life. Of older agents only 5-FU and mitomycin C have consistently shown to have any beneficial effect (*Cullinan et al. 1990; Haycox et al. 1998b; Maisey et al. 2002*). Although the survival benefit from gemcitabine is small in absolute terms compared with bolus 5-FU it is accepted as the standard drug for treatment of non-resectable or metastatic pancreas cancer. Chemotherapeutic combinations or radiochemotherapy has no proven additional effect on survival (*Neoptoleomos et al. 2004; Cheverton et al. 2004; O'Reilly et al. 2004; Richards et al. 2004; Louvet et al. 2004*).

There is an urge to seek out different strategies for treatment of pancreas cancer. Intraperitoneal chemotherapy is such a strategy.

5. Background

Pancreas cancer

Epidemiology

All cancer types in Sweden have been nationally registered since 1958. There is an annual increase of primary cancer incidence of around 2%. The incidence of pancreas cancer (10.1 for men and 9.2 for women in 2002) is slightly falling, resulting in less than 900 new cases each year (*The national board on health and welfare 2002*). Cancer of the pancreas is globally one of the leading causes of all deaths in cancer. The incidence in the world is widely spread. In Herault, France it is 3.1 and in Louisiana, USA 20.8 in the Afro-American population. The male/female ratio in industrial countries is 1.6, but the gap is closing between men and women to the extent that women is over-represented in ages 50-60 years. Cigarette smoking increases the risk of pancreas cancer 2-fold but increases the risk, as a comparison, of lung cancer 20-fold. The relationship between diet and pancreas cancer remains unclear, but obesity and diet with high fat content is a risk factor for acute pancreatitis and pancreas cancer (*Silverman et al. 1998; Chowdhury and Rayford 2000; Cavestro et al. 2003; Farrow et al. 2004; Michaud et al. 2001*). The relationship between pancreas cancer and diabetes is controversial. Diabetes may manifest simultaneously with pancreas cancer without giving an increased risk of developing the diseases (*Yalniz and Pour 2004*). In some genetic syndromes, pancreas cancer has been found more common (*Hruban et al. 1998*).

Clinical features

The symptoms of pancreas cancer are insidious, vague, and easy to misinterpret, and patients often wait to seek medical attention until jaundice or abdominal pain is overwhelming. As summarised in Table 1, the most common symptoms are weight loss, often more than 10 kg, and diffuse abdominal pain. If the cancer is located in the body of the pancreas, jaundice is an uncommon presenting symptom (< 7%).

Table 1 Presenting symptoms in pancreas cancer

	Warren	Bakkevold	Klinkenbijl
Weight loss	90%	58%	77%
Abdominal pain	80%	72%	70%
Jaundice	75%	47%	71%
Nausea, emesis	30%		30%
Diabetes without heredity	5%		
Back pain	40%		
Acute pancreatitis	3%		

Legends: Warren et al. 1983; Bakkevold et al.1992; Klinkenbijl et al.1993

In the normal pancreas gland the acinar cells, elaborate in production of digestive enzymes, stands for 90% of the organ volume, and ductal cells, responsible for the secretion of fluids and electrolytes and the conveyance of the pancreatic juice to

the duodenum, for 1% of the volume. In cancer of the pancreas 90% are ductal adenocarcinomas and only 1% acinar cancers.

Surgical treatment

The only chance to cure pancreas cancer is surgical resection, most commonly performed as a pancreatico-duodenectomy, also known as the "Whipple's procedure" (*Whipple 1935*). Less than 20% of patients do have resectable disease, leaving the remainder with either locally advanced disease or metastatic pancreas cancer at presentation. Locally advanced pancreas cancer is defined as an non-resectable tumour without evidence of distant metastases. A tumour is considered non-resectable if it has either extensive peripancreatic lymph node involvement or severe involvement of the superior mesenteric vein (SMV), portal vein (PV), superior mesenteric artery, inferior vena cava, aorta, or celiac axis. If it is possible to do an en-bloc resection of the SMV/PV confluence for tumour extension in this area, the patients do not seem to have worse survival compared with patients with less extensive disease. (*Capussotti et al. 2003*)

Patients judged resectable on CT and MRI are prior to resection, laparoscopically evaluated for metastases in the liver and peritoneum. Superficial peritoneal dissemination develops early, and up to 30% of patients with apparent localised disease exhibit metastases (*Pisters et al. 2001*). Peritoneal washing, endoscopic ultrasound and percutaneous ultrasound-guided fine-needle aspiration cytology may be used for biopsy and pathologic diagnosis. Positive cytology in abdominal lavage can predict a higher failure rate of resection (*Warshaw et al. 1991; Leach et al. 1995*).

The patterns of failure are no more than a reflection of the extent of the disease at diagnosis. Local recurrences of the cancer occurred in the bed of resection (50-73%), in peripancreatic and regional lymph nodes (63-86%), as liver metastases (50-62%) or on the peritoneal surfaces (42%). Extra-abdominal metastases are found in 27% (*Griffin et al. 1990*). The failure to optimally treat each involved region will result in the ultimate demise of the patient.

Adjuvant therapy

Radiotherapy can be delivered as external beam radio therapy (EBRT) or intra operative radiation therapy (IORT) and is like surgery a locally directed treatment, unable to deal with all intraabdominal sites of recurrence. The tolerance of radiation for normal tissue in the upper abdomen is 45-54 Gy, given in 25-30 fractions. IORT has the ability to deliver a more focused and intense dose than EBRT to the pancreas resection bed, the site of most recurrences. Although it is proposed, that IORT may improve local control in some patients with small resectable tumours, distant failure prevents any advantage in survival (*Schwarz et al. 2003; Alfieri et al. 2001; Sindelar and Kinsella 1999*).

The rationale for adjuvant chemoradiation following tumour resection therapy is the high incidence of both local and distant tumour recurrence. The radiosensitation

by concomitant 5-FU therapy was established by Moertel in 1969 (*Moertel et al. 1969*). Patients with advanced pancreas cancer demonstrated an improved median survival with a combination of EBRT plus 5-FU when compared with EBRT alone (10.4 versus 6.3 months, respectively). Several studies have later unsuccessfully tried to establish the superiority of chemoradiotherapy in the adjuvant setting (*Klinkejbijl et al. 1999; Neoptolemos et al. 2004*).

The most efficient doses and schedules of radiation in combination with 5-FU or gemcitabine after resection are explored in several studies. Dose limiting toxicity occurred at 39 Gy with concurrent gemcitabine (*Allen et al. 2004*). In other schedules 55 Gy was delivered with 5-FU as continuous infusion and found feasible (*Balosso et al. 2004*). The final results of the ESPAC-1 study (*Neoptolemos et al. 2004*) advocates that adjuvant chemoradiotherapy had a deleterious effect on survival (median survival 13.9 months) whereas adjuvant chemotherapy with 5-FU and Leucovorin IV, had a significant survival benefit in patients with curatively resected pancreas cancer (median survival 21.9 months). The ongoing ESPAC-3 study compares, after closing the observational arm, 5-FU and gemcitabine as adjuvant chemotherapy after macroscopically curative resection.

Palliative therapy

The major aim of chemotherapy is to deliver cancercidal doses of drugs to obtain effective tumour cell destruction with minimal damage to non-cancerous tissue. The poor results in the treatment of patients with advanced metastatic gastrointestinal cancer may partly be explained by the difficulty of intravenously administered drugs to reach routes of metastatic dissemination such as the peritoneal cavity and retroperitoneal lymph nodes (*Schiessel et al. 1989; Ettinghausen et al.1995*).

Cancer of the pancreas exhibits an exceptional resistance to chemotherapy. One proposed contributing factor is the high expression of the multidrug resistance gene (MDR1) product, P-glycoprotein. This membrane protein is a part of a drug and toxin efflux enzyme system that rapidly clears tumour cells of many cytotoxic drugs, as adriamycin, vincristine, etoposide, pacitaxel and mitomycin C (*Lage and Dietel 2002*). 5-FU is not affected by the P-glycoprotein. About 85% of all pancreas cancers express a K-ras oncogene mutation (*Capella et al. 1991*) yielding increased cellular proliferation. A p53 tumour suppressor gene mutation is expressed in 50% of pancreas cancer (*Scarpa et al. 1993*). The MDR1 gene could be activated during tumour progression associated with mutations in p53 (*Chin et al. 1992).*

The dihydropyrimidine dehydrogenase (DPD) enzyme, is the first step in the catabolism of 5-FU. DPD activity was found to be higher in pancreas cancer than in normal pancreas tissue (*Nagakawa et al. 2000*). High DPD activity in the tumour is thought to make the tumour less sensitive to fluorinated pyrimidines by reducing the amount active metabolites of 5-FU.

The average response rates for pancreas cancer with systemic chemotherapy range from 7 to 28% using 5-FU based regiments (*DeCaprio et al. 1991; Choi et al. 2000*). Different ways of administration, as bolus injection for 2-5 minutes, short or long-time infusions, give different plasma AUC and Cmax. Protracted venous infusion with 5-FU 300 mg/m^2 per day has shown to give very low toxicity (*Maisey et al. 2002*).

Gemcitabine has produced response rates of 10-15% in patients with advanced pancreas cancer. A study, in which the patients were given gemcitabine, focused on improvement of disease-related symptoms measured as "clinical benefit score", a criteria designed by that study group (*Burris et al. 1997*). The advantages with gemcitabine versus 5-FU measured by use of the clinical benefit score, were statistically significant, and thereon gemcitabine is commonly used in palliative chemotherapy. Gemcitabine has been tested in combination with several chemotherapeutic agents as 5-FU, mitomycin C, tipifarnib, docetaxel, cisplatin and oxaliplatin (*Oliani et al. 2004; Kanat et al. 2004; Tuinmann et al. 2004; van Cutseem et al. 2004; Novarino et al. 2004; Louvet et al. 2004*). None of these regimens resulted in significant better survival time, but all gave WHO grade 3 and 4 toxicity to 24-54% of the patients.

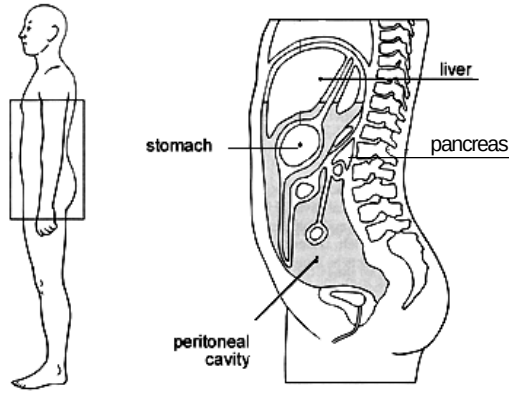
Combinations of chemotherapeutic agents have occasionally led to claims for high response rates (and increased toxicity), but these results have not been confirmed in more extensive follow-up studies. The difficulty to reproduce initially hopeful results in larger studies, seems to be a general pattern in chemotherapy against pancreas cancer.

Intraperitoneal chemotherapy

The use of the peritoneal cavity for chemotherapy administration is based on the physiological mechanism of transport between blood and the peritoneal space. The peritoneal dialysis system consists of the peritoneal cavity which contains the fluid, the peritoneal membrane including the highly permeable mesothelium, the interstitium that surrounds blood vessels, and the peritoneal microvasculature. The capillary fluid absorption and the lymphatic fluid absorption are the determinants for the loss of fluid from the peritoneal cavity. The capillary wall is the major exchange barrier between blood and the peritoneal cavity (*Rippe et al. 2001*).

The capillaries present a continuous type endothelium in which endothelia cells are connected by tight junctions. The transcapillary exchange route for water and small solutes is across the capillaries and postcapillary venules through a protein restrictive pathway between the endothelial cells. This pathway has a functional radius of 40-50 angstrom and accounts of 99% of total exchange (pore) area. Lymphatic absorption is very low, only 0.2-0.3 ml/min (*Rippe and Zacharia 1992; Rippe and Haraldsson 1994).*

Figure 1 The intraperitoneal space



In 1955, the result of intraperitoneal nitrogen mustard treatment of patients with ovarian cancer was reported (*Weissberger 1955*). Although the control of malignant ascites was ample, the toxicity was substantial and no considerable effect on the intraabdominal tumour masses was demonstrated. For more than two decades, the intraperitoneal (IP) route was rarely employed. In 1978 a pharmacokinetic rational for intraperitoneal drug therapy in the treatment of ovarian cancer was presented (*Dedrick et al. 1978*). Cytotoxic drugs were cleared much more rapidly from the systemic circulation, than from the peritoneal cavity. Intraperitoneal administration resulted in a concentration gradient across the peritoneal membrane, with higher concentrations in the peritoneal cavity relative to blood. Several studies have demonstrated very high peritoneal to plasma ratios for adriamycin, cisplatin, melphalan, 5-FU, mixantrone, FUdr, 9-aminocamptothecin, topotecan, oxaliplatin and docetaxel (*Ozols et al. 1982; Howell et al. 1984; Gywes et al. 1984; Nicoletto et al. 2000; Muggia et al. 1991; Muggia et al. 2002; Hofstra et al. 2002; Elias et al. 2002; Morgan et al. 2003*).

A study comparing two routes of administration, intravenous vs. intraperitoneal cisplatin 100 mg/m^2 plus IV cyclophosphamide in patients with ovarian cancer stage III, demonstrated a significant survival advantage and less systemic toxicity for the intraperitoneal route (*Alberts et al. 1996*).

The feasibility of intraperitoneal 5-FU in combination with intraperitoneal cisplatin was explored in a study by Schilsky (*Schilsky et al. 1990*) where patients with intraabdominal malignancies were given 90 mg/m^2 cisplatin mixed with 5-FU in increasing doses from 1300 mg to 5200 mg in 2000 ml lactated Ringers solution every twenty-eighth day, with dose limiting neutropenia occurring at a 5-FU dose of 2600 mg/L. There are some studies of intraperitoneal chemotherapy against pancreas cancer, none with intention to treat, showing a substantial increase of total drug exposure, i.e. AUC (area under the concentration curve x time) for peritoneal fluid compared to plasma. In a phase II study with intraperitoneal Citrovorum

factor 50 mg followed by intraperitoneal 5-FU 1000–3400 mg as a single weekly dose, the side effects were few with only one event of myelosuppression. No myelosuppression was noted in patients receiving 1200 mg/m^2 or less (*Budd et al. 1986*). In a series of 12 patients with intraabdominal tumours (9 with pancreas cancer) receiving intraperitoneal 5-FU 2 – 4 mmol/L and intraperitoneal Leucovorin in 2000 ml saline. The maximal peritoneal to plasma AUC ratio was 461 at the 2 mmol/L dose, but decreased with increasing doses as systemic clearance decreased (*Arbuck et al. 1986*). In portal blood Cmax was 29–31 mg/L at 8–11 minutes and AUC was higher than in peripheral blood after 15 mg/kg 5-FU IP (*Kakizaki et al. 1993*).

The overall efficacy of the therapy may be compromised if systemic drug delivery is inadequate with less drug being delivered to the tumour through the capillary system. To fully use the intraperitoneal route up to the systemic dose limiting toxicity, the systemic exposure after intraperitoneal treatment should be as high as achieved with intravenous administration of the drug. The local effect of the drug in the peritoneal cavity, is therefore of great importance for its systemic anti-tumour effect. If the dose limiting toxicity of intraperitoneal drugs are local, the systemic exposure supposedly is less than attained with intravenous administration. In this aspect, it is crucial to chose a drug with low local toxicity. Drugs like mixantrone and mitomycin C have high local toxicity, and therefore reduced ability of generating systemic effects after intraperitoneal administration (*Alberts et al. 1988; Monk et al. 1988*).

Drugs like 5-FU and cisplatin have low local toxicity, with good chances of yielding adequate high systemic concentrations thus affecting the tumour through the capillary system. Intraperitoneal 5-FU gives a peritoneal to plasma AUC ratio in the range of 117 to 1066 depending on the dose schedule used (*Speyer et al. 1980; Speyer et al. 1981; Gyves et al. 1984; Sugarbaker et al. 1990*) and is accompanied by high concentrations of the drug in the lymphatics measured at the level of the diaphragm, as shown in a pig model (*Lindner et al. 1993; Lindner et al. 1996*). The highest concentration of the drug might be anticipated in the lymphatic drainage closest to the peritoneal surface. More than 85% of intraperitoneally administered fluoropyrimidine is eliminated by the liver in the first passage effect.

Peritoneal blood flow measurements

The peritoneum has a relatively large surface area in adult man, around 0.5 x body surface area and it is composed of the parietal part (10–15%) and the visceral (including omental and hepatic) part (85–90%). Total splanchnic blood flow is 1200 ml/min at rest, but only 70–100 ml/min comes in contact with the peritoneum (*Aune 1970; Bulkley 1981; Grzegorzewska and Antoniewicz 1993)*. Due to deficient contact or poor mixing of fluid in small compartments, there is a limitation of exchange across the visceral surfaces.

Measuring continuous blood flow changes in an intact peritoneal cavity poses some considerations. The registration should not require measurement of another parameter that will be less accessible, the result should not be affected by other factors than blood flow (as permeability and surface area), and the application itself should not cause alterations of blood flow (as could be the case for extensive surgical methods and electrode placements).

The electromagnetic flow probe detects distortion of a magnetic field across the vessel, generating an electric field, proportional to the velocity of blood. The ultrasonic volume flow probe emits ultrasound that changes in frequency as it traverse the blood vessel measuring velocity of flow. Both these methods require the placement of a flow probe around a specific vessel and are therefore less suitable for peritoneal blood flow measurements.

Microspheres are small solid particles that will be trapped in the region of interest proportional to the regional blood flow. Their distribution depends on the size of the spheres and the diameter of the vessels (*Phibbs and Dong 1970*). Reaching the capillary bed, they must be trapped without interfering with the microcirculation, a situation that can turn into non-entrapment and interference as the tumour vascular bed has arterio-venous shunts, abnormally wide capillaries and different flow patterns (*Naredi and Hafström 1992*). The method requires sampling of tissue and does offer repeated but not continuous monitoring of blood flow.

Laser doppler flow

In 1842 Johan Christian Doppler delivered his thesis “Über das farbige Licht der Doppelsterne” to the Royal Bohemian Society of Learning. Since then, the Doppler principle that the frequency of the radiation scattered by a moving object is changed depending on the velocity of the object and the scattering geometry, has been utilised in many scientific disciplines. The first time the Laser doppler method was used to measure blood flow was in 1972 (*Riva et al. 1972*). The Laser doppler flow probes emits an infrared laser light signal, that passes across the tissue and is backscattered by moving blood to a photo detector. The method, has the drawback of being invasive, but the clear advantage of measuring blood flow in a region, rather than in a specific vessel.

Inert gas clearance techniques

Kety (*Kety 1951*) predicted hydrogen to have the potential as a blood flow marker, as it is metabolically inert and not normally present in the body. Hydrogen gas has a low recirculation, a low water-gas partition coefficient, and a high diffusibility in the tissues. Gas clearance studies assume that gas clearance is equivalent to effective blood flow (*Aune 1970*). Hydrogen clearance can be made repetitively but the effect of insertion of electrodes in the tissue can cause tissue damage, thus interfering with the measurements.

Noble gases express many ideal exhibits for the purpose of minimal-invasive repetitive blood flow measurement. They diffuse rapidly into the tissues so that an instant equilibrium between blood and tissue is obtained. Noble gases are momentarily cleared by the lungs with virtually no recirculation and they are physiologically inert. Xenon-133 (^{133}Xe) is a commercial available inert lipid-soluble noble gas with a T½ of 5.2 days. The gamma emission of ^{133}Xe is easy to shield and well suitable for external imagining. The disappearance can be continuously monitored with a scintillation detector or a gamma camera positioned over the organ. Scintillators respond to gamma-rays as well as neutrons. Hence a gamma-ray induced background count rate will exist in these build-up monitors, but this background bias can be handled with pulse height discrimination, which rejects events that fall outside the peak region. Sodium iodide detector activated by thallium (NaI:Tl) has long been the scintillation standard. It has a high luminescence efficiency, good spectroscopic performance and no significant self-absorption of the scintillated light.

Conventionally the radionucleotide ^{133}Xe in gas form is inhaled or delivered to the organ of interest through an intra-arterial, intra-portal or intra-parenchymatous injection in an aqueous solution. Injected in closed compartments this technique was introduced for the evaluation of peritoneal perfusion and the detection of intestinal ischemia. In a rat experiment, intraperitoneally introduced ^{133}Xe showed a significant delay in washout when the strangulated bowel was ischemic (*Bulkley et al. 1981*). Radioactive ^{133}Xe, upon entering the peritoneal cavity rapidly distributes throughout the abdomen, diffuses across the peritoneal surface into the underlying exchange vessels, and is carried by the blood to the lungs where it is eliminated to about 95% at its first passage (*Bulkley 1981*). The recirculation is negligible and the isotope is found in its highest concentrations in the expired air. The expired air is evacuated to prevent build-up of background radioactivity in the laboratory room.

Aiming at a minimal-invasive method with the possibility of sequential measurements before and during intervention, the ^{133}Xe-clearance technique seemed to be applicable to estimate the peritoneal blood flow changes of vasopressin.

6. Aims

Our hypotheses are that 5-FU administered via the intraperitoneal route, is a safe treatment against non-resectable pancreas cancer and that intravenous vasopressin improves 5-FU pharmacokinetics.

The overall aim was to investigate the feasibility and efficacy of intraperitoneal 5-FU in treatment of patients with non-resectable pancreas cancer, using vasopressin to improve the pharmacokinetic profile. Further we wanted to study the effect of vasopressin on peritoneal blood flow, altered by the therapy itself or by the presence of peritoneal carcinomatosis.

Specific aims

Experimental studies

To investigate if the ^{133}Xe-clearance technique identifies changes of peritoneal blood flow caused by intravenous vasopressin.
To explore the effect on peritoneal blood flow of intraperitoneal 5-FU administration and of peritoneal carcinomatosis.
To explore whether peritoneal blood flow reacts to intravenous vasopressin in intraperitoneal 5-FU treated rats, or in rats with peritoneal carcinomatosis.

Clinical studies

To evaluate the safety, toxicity and tumour response of escalating doses of intraperitoneal 5-FU and intravenous Leucovorin treatment in patients with non-resectable pancreas cancer.
To monitor the pharmacokinetics of 5-FU in plasma during intraperitoneal 5-FU installation with and without concomitant intravenous vasopressin infusion.

7. Materials and methods

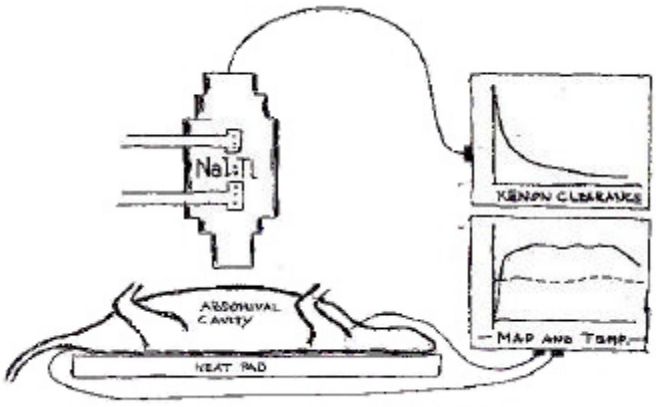
Animals and preparation

Inbred Wistar-Fu (W-Fu) rats and Lister-Hooded (LH) rats of both sexes were used in the experiments. The animals were maintained on a standard pellet and water diet at a day and night 12-hour rhythm.

All handling and measurements of the animals were performed while animals were under anaesthesia with an intraperitoneal injection of 3 ml/kg bodyweight of midazolam (5 mg/ml), fentanyl citrate (0.3 mg/ml) and fluanisone (10 mg/ml) diluted in sterile water (Jansen Animal Health, Belgium). At conclusion of the experiment, the rats were sacrificed with an intravenous injection of Pentobarbital sodium.

Body temperature was maintained by placing the animal on a heat pad. Rectal temperature was registered by a rectal probe connected to a thermal register. Heat pad temperature was regularly adjusted during the experiment to maintain a body temperature well above 36.0 °C. Mean arterial blood pressure was registered via a catheter in the external carotid artery.

Figure 2 Set up of ^{133}Xe-clearance experiment



Experimental tumours

A 3-methyl-diaminobenzidine-induced syngeneic hepatoma (Hep) in Lister-Hooded rats obtained from the Chester Beatty Research Institute (*Kjartansson 1976*), and a N-methyl-N'-nitrosoguanidine-induced syngeneic adenocarcinoma of the colon (NGW) in Wistar-Fu rats from the Wallenberg Research Laboratory in Lund (*Steele 1974*), were used. These two experimental tumours were chosen as they have been studied in different tumour models in our laboratory (*Hafström et al. 1980; Naredi et al. 1993*). The tumour take and growth rate are satisfactory reproducible.

The tumours were maintained viable by passage transplantation every 12 to 14 days. For experiments, fresh tumour tissue were homogenised in saline. Vital cell counting was performed in a Bürkner chamber with added Nigrosin 0.3% solution (Sigma-Aldrich Sweden AB, Stockholm, Sweden). To achieve peritoneal carcinomatosis 10^5 viable tumour cells were injected intraperitoneally.

In our implantation model the tumour take frequency was more than 85%. The tumour growth was considerably variable, from a few nodules on the parietal peritoneum to an almost complete coverage of the peritoneum and omentum. Animals with no tumour take were excluded from further experiments. There was no obvious difference in tumour take or growth between the two tumour types. The result of the ^{133}Xe-clearance was not known when tumour mass was scored.

Blood flow measurements

^{133}Xe-clearance technique

Ten to 50 microliter ^{133}Xe (10-15 MBq) was promptly injected percutaneously into the abdominal cavity. ^{133}Xe activity was registered with a well collimated NaI:Tl - scintillation detector connected to a multichannel analyser (Figure 2). Pulse height discrimination was used and the energy window was set at 30 keV symmetrically around the 81 keV gamma peak from ^{133}Xe, and incoming pulses were recorded in 10-second intervals.

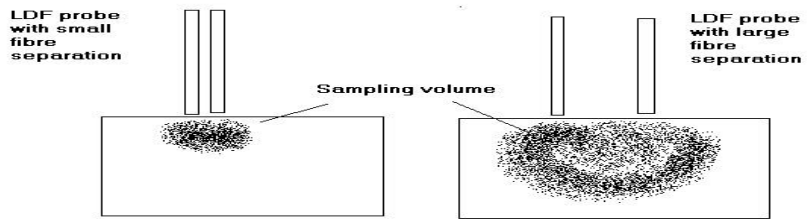
The initial pulse rate was 300–500 counts per second (CPS) and the background radiation was 3–7 CPS and hence negligible. The endpoint of the analysis was chosen at 600 seconds after injection of ^{133}Xe since the k-values in this part of the curve were close to zero. After recording the basal ^{133}Xe-clearance, 0.007, 0.07 or 0.14 IU/kg/min of vasopressin (Ferring, Malmö, Sweden) was infused into the tail vein with an infusion pump (Terumo Syringe Pump, model STC-521, Tokyo, Japan) for 10 minutes, and an identical registration was performed.

Corresponding distribution images with a gamma camera (Siemens ECAM Single head, Siemens AG, Münich, Germany) able to analyse the activity in the whole rat or specifically in other regions of interest were acquired. The rats were placed directly on the medium energy high resolution (HiRes) collimator and injected IP with 10–15 MBq of ^{133}Xe. Counts for ^{133}Xe-clearance were analysed from an abdominal area with 6 cm in diameter.

Laser doppler flow

The vasopressin effect on peritoneal circulation was also assessed with a Laser doppler probe with a separation of fibres of 0.25 mm (Probe 404, Perimed AB, Järfälla, Sweden). This separation yields a small volume of tissue where the blood flow is recorded, as the depth of sampling is approximately 0.5–1.0 mm. Greater separations yields larger sampling depth (Figure 3).

Figure 3 Illustration of probe separation and sampling depth. A wider separation gives a deeper sampling volume.



After performing a midline laparotomy on the rat, the probe was placed in a dissected pocket of peritoneum turned towards the abdominal contents. Between the peritoneal sheet and the bowels, a thin black plastic sheet was placed to minimise artefacts from organ circulation in the abdomen. The skin was closed over the probe cable, which was connected to a PeriFlux 4001 Monitor. The incoming signals were sampled at 64 Hz with a time constant of 0.03 seconds and recorded every 30 seconds. After baseline registration of LDF for ten minutes, 0.07 IU/kg/min of vasopressin was administered IV. Registration was continuous, with the exception for brief extrication and irrigation of the probe with isotonic saline to prohibit clotting of debris impairing the signal.

The correlation between ^{133}Xe-clearance and LDF has been addressed, mainly in estimations of skin perfusion and circulation in muscle (*Olsson 1986*; *Monterio et al. 1989*)

Compartmental analysis
Compartmental analysis is the mathematical resolution of complex inert gas washout curves into simpler component parts, reflecting a physiologic phenomenon. By assuming that the tissue is homogenous, contains no concentration gradients of the tracer, the equilibrium between tissue and blood is immediate (seconds or less) and the isotope is removed only by blood flow from the site sensed by the external detector, the equation is:

$$dq(t) / dt = (-F / \lambda \times V) \times q(t)$$

q = quantity of tracer remaining at the site, t = time, F = blood flow, V = volume of the tissue, λ = constant expressing the ratio of the concentration of tracer in the tissue at equilibrium to its concentration in effluent blood flow (*Kety 1951*). The concentration of Xenon in the venous blood leaving the organ is therefore in continuous equilibrium with the tissue concentration. The gas is transported from the tissue at a rate that is proportional to the blood flow. Repeated measurements are possible as elimination through exhaled air is almost complete, and thus the recirculation is very low. The mathematical model for analysis of ^{133}Xe-clearance curves can be regarded as a system of two parallel compartments where the

washout can be expressed as a bi-exponential equation:

$$A(t) = C_1 e^{-k_1 t} + C_2 e^{-k_2 t} \quad (1)$$

A(t) is the ^{133}Xe activity in the organ at time t and C_1 and C_2 are coefficients calculated from the initial conditions in each compartment. The coefficients k_1 and k_2 are the elimination rates of the two compartments. In this case, the whole curve, 600 seconds, was analysed. The fast phase (k_1) is dominating the first part and the slow phase (k_2), the last part of the curve. The two compartmental model is considered to be a simplification of a much more complex function of clearance of ^{133}Xe (*Bolmsjö 1983*). It is possible to use an even more simplified compartment model consisting of one single compartment (*Watson and Cloutier 1977; Young et al. 1988*) where the washout of the ^{133}Xe (k_3) can be expressed as:

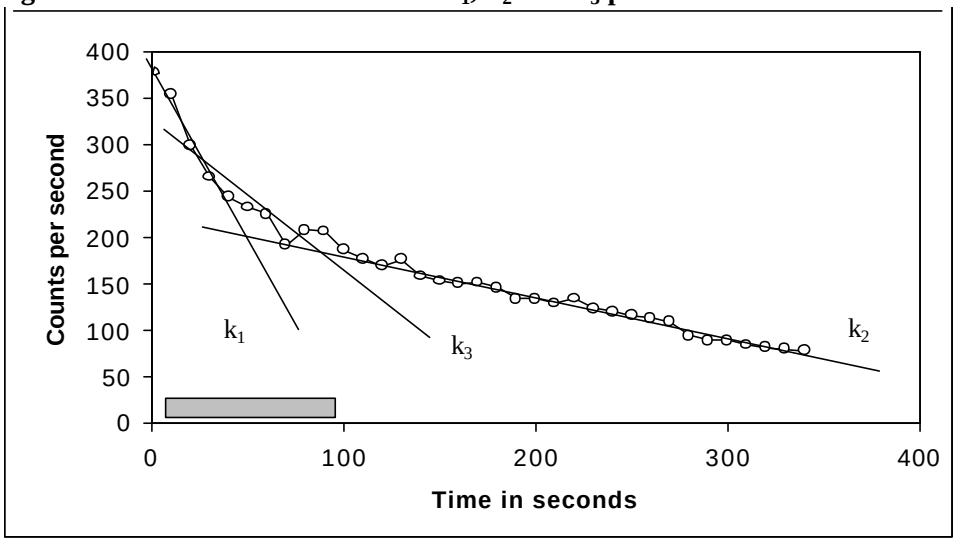
$$A(t) = C_3 e^{-k_3 t} \quad (2)$$

In this single compartment analysis, only the first 90 seconds (from 10 to 100 seconds) of the curve was analysed. By mathematical manipulation of equation (1) and (2), it can be showed that:

$$k_3 = -\ln \left(C1 / C1 + C2 \times e^{-k_1 t} + C2 / C1 + C2 \times e^{-k_2 t} \right) \quad (3)$$

It should be noted that this approximations is valid only in the initial part of the washout curve. The retention in peritoneal fat, where xenon is highly soluble (λ= 10), as compared to intestinal tissue (λ=0.67), will produce a delayed tail in the washout curve, thereby most affecting the slow compartment. The monoexponential curve avoids that area of the curve.

Figure 4 ^{133}Xe-clearance curve with k_1, k_2 and k_3 plotted



Legends: k_1 and k_2 fast and slow clearance in bi-exponential analysis and k_3 clearance in the mono-exponential analysis. Filled rectangle indicates time during which k_3 is derived.

In the mathematical analysis, the different k-values were calculated by fitting equations (1) or (2) to the measured data. The equations were fitted to the washout curves using a method by minimising the deviations between the acquired data and the fitted equation. The computer algorithm uses the method of least squares, which is based on the hypothesis that the optimum description of a set of data is one that minimises the weighted sum of squares of deviations of the data, from the fitting function. This sum is characterised by a variance of the fit performed by calculation of:

$$\chi^2 = \sum_{i=1}^{N} (\overline{x}_i - x_i)^2 \qquad (4)$$

where, $\overline{x}_i$ denotes the fitted data and x_i denotes the measured data. By minimising the χ^2 by the method of maximum likelihood an optimal fitting function is obtained (*Bevington 1969*). All fitting calculations were done with the Origin 6.1 Graph and Data Analysis program (Origin Lab Corporation, Northampton, MA, USA).

With the concentrations of ^{133}Xe in the tissue and blood at equilibrium, i.e. the partition coefficient (λ), the absolute blood flow (ml/min/g tissue) can be estimated as the product of λ and k. The high lipid-solubility of ^{133}Xe, arises the possibility of entrapment of isotope residues in the fat in and around the peritoneal cavity. As calculation of λ must take this heterogeneous tissue in account, it was not calculated. The aim was to register only relative changes of blood flow.

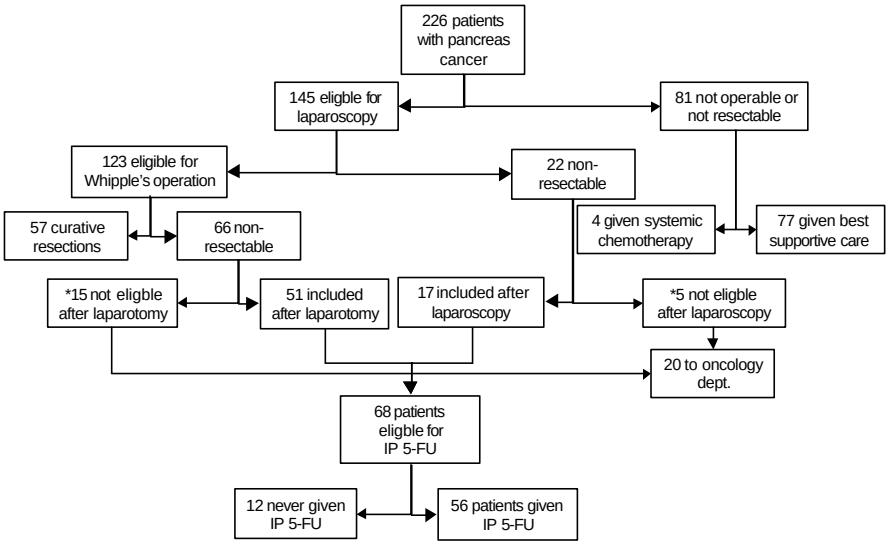
Intraperitoneal 5-Fluorouracil

Patients

Two-hundred and twenty-six patients with ductal pancreas carcinoma were admitted to the department of surgery, Umeå university hospital, between 1994 and 2003. After evaluation with a computer tomographic scan (CT), a magnetic resonance image (MRI) and laparoscopy, 123 patients were intended to a curatively aiming pancreatico-duodenectomy. The selection of patients is depicted in Figure 5.

In total, 68 (29 men/39 women) patients, with a morphologically or cytologically documented non-resectable or metastatic ductal pancreas carcinoma were included. These patients were required to have a Karnofsky Index (*Karnofsky and Burchenal 1949*) of 70 or more and an abdominal cavity free of extensive adhesions. Patients were consecutively allocated to the different groups, beginning with 750 mg/m^2, with the aim of 12-18 patients in each group. Baseline patient characteristics are listed in Table 2.

Figure 5 Patients recruited for intraperitoneal 5-FU



Legends: * additional renal cell cancer (n=1), histopathology not conclusive (n=6), abdominal adhesions (n=3), referring hospital could not provide treatment (n=2), choice of the attending surgeon (n=8).

Table 2 Baseline patient characteristics

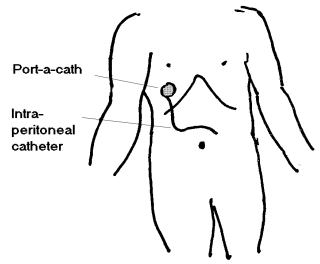
	All n=68	**0 mg n=12**	**750 mg n=18**	**1000mg n=12**	**1250mg n=12**	**1500mg n=14**
Men/Women (n)	29/39	4/8	9/9	4/8	5/7	7/7
Age*	62 (36-89)	65 (53-76)	63 (36-73)	69 (45-89)	57 (47-76)	57 (46-75)
Operation						
Laparotomy	51	7	13	9	12	10
Laparoscopy	17	5	5	3	0	4
Tumour						
Size < 3 cm	14	2	2	2	1	7
Size ≥ 3 cm	41	7	12	5	10	7
NA	13	3	4	5	1	0
Metastases						
Liver	23	5	4	7	2	5
Peritoneum	20	4	6	5	1	4
Differentiation						
High	11	2	5	1	2	1
Intermediate	49	7	13	7	10	12
Low	8	3	0	4	0	1
TNM stage						
III	14	2	1	2	6	3
IV	54	10	17	10	6	11

Legends: Number of patients.* Age in years median (range). NA = not assessable on first CT scan.

Surgical procedure

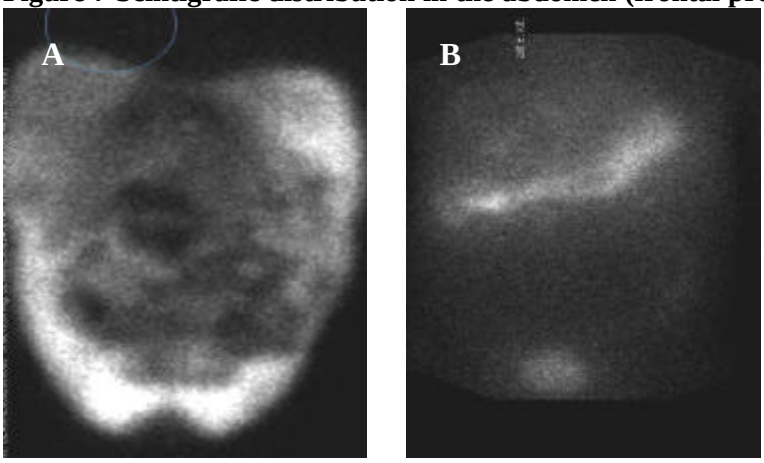
A Port-a-cath (PAC, JCL Technic, Vallentuna, Sweden) was applied at laparotomy (n=51) or laparoscopy (n=17). In two patients the PAC was placed at laparotomy on the abdominal wall below the subcostal incision, instead of over the costal edge. The technique was abandoned because introducing the infusion needle was considerably more difficult, in spite of the thin subcutaneous adipose tissue on the abdominal wall. The PAC was on all other patients placed subcutaneously over the right costal edge and the catheter floating free in the abdomen (Figure 6). When the PAC was laparoscopically placed, effort was made to pass the catheter through the abdominal wall in a soft curvature, avoiding 90 degree angle that could impede infusion and promote leakage.

Figure 6 Catheter and port placement over right costal edge



The IP distribution of the instillated drug was controlled by Technetium-99 scintigraphy before starting treatment and then every 3 months. Activity in all four quadrants of the abdomen were considered as adequate for delivering cytostatics to the major part of the peritoneal cavity (Figure 7). Inclusion date was set to the date of implanting the PAC.

Figure 7 Scintigrafic distribution in the abdomen (frontal projection)



Picture A: Activity in all four quadrants of the peritoneal cavity.
Picture B: Activity in the upper part of the peritoneal cavity.

Treatment schedule

The treatment was administered for two consecutive days every third week. The first treatment was given in hospital and the following on an out-patient basis. Ondansetron 8 mg IV was given shortly before chemotherapy, and then 4 mg bid orally for two to three days. 5-FU 750–1500 mg/m^2 body surface was administered intraperitoneally by gravity during 30–60 minutes. Thirty minutes after the start of the 5-FU infusion, Leucovorin 100 mg/m^2 body surface was given as a slow intravenous injection. The treatment was repeated in the same way the next day. There was no drainage of fluid after treatment.

At every treatment, performance status with Karnofsky index (KI) (*Karnofsky and Burchenal 1949)*, morphine consumption and weight were recorded. Laboratory values were assessed 10–16 days after and immediately before treatment. Every third month, along with clinical assessment and pulmonary X-ray, tumour response was evaluated by CT scan according to the WHO criteria.

Clinical evaluation

Tumour response was registered as time to progression and survival time. Time to progression was defined as the time from operation with PAC placement to the first objective documentation of tumour progression, or to the time of death in the absence of previous documentation of objective progressive disease. Survival was defined as the time from operation to the date of death.

Safety evaluation parameters included assessment of laboratory values for haematological, renal and hepatic functions every other week, and assessment of adverse advents at every treatment.

Stable performance status during treatment was defined as: no worsening of the Karnofsky Index, less than 10% increase in morphine consumption, and a decline in weight of less than 5% from baseline.

Pharmacokinetic study

In seventeen patients, receiving 750 mg/m^2 intraperitoneal 5-FU, an intravenous infusion of vasopressin 0.1 IU/minute was given during 180 minutes. The infusion was given alternatively day 1 or 2, with start immediately before 5-FU instillation. Blood samples to determine 5-FU plasma concentration were drawn every 30 minutes either from 0 to 180 minutes (432 samples) or from 210 to 390 minutes (82 samples), and then centrifuged. The plasma and peritoneal fluid were frozen at -70 °C until analysed. The concentration of 5-FU was measured in deproteinized plasma samples by high performance liquid chromatography (HPLC) (*Gustafsson 1979*).

The peritoneal fluid 5-FU content was not measured because patients rapidly developed ”one-way-valves”, making it difficult to obtain sufficient amounts of fluid through the small size catheter. Shifting to a larger size catheter was not done, as the PAC was readily accepted by the patients.

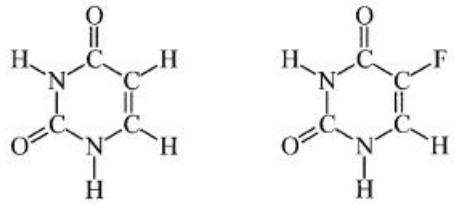
In order to explore if the sampling procedure could have impact on the 5-FU concentration measurements, one patient receiving 1500 mg/m^2, was subjected to collection of double blood and peritoneal fluid samples. One sample was immediately centrifuged and frozen, and the corresponding sample was left in room temperature for two hours before handling.

Drugs

5-Fluorouracil

The antimetabolite 5-fluorouracil (5-FU), is a small molecule (molecular weight 130.08), synthesised in 1957 (*Heidelberger et al 1957*), by replacing a hydrogen with a fluorine atom at the carbon-5 position of the pyrimidine ring (Figure 8). As an uracil analogue, it serves as a substrate for the same substrates in transport and metabolisation.

Figure 8 Uracil and 5-Fluorouracil



5-FU requires cellular uptake and metabolic activity in order to exert cytotoxicity. 5-FU enters the cell both by diffusion and by an active transport system. Normal cells and tumour cells metabolises 5-FU to 5-fluoro-2´-deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). These metabolites cause cell injury as antagonists to pyrimidine by two different mechanisms. First, the production of DNA is hampered because FdUMP binds to thymidylate synthetase (TS), an enzyme crucial in the chain of DNA generation. Second, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate (UTP) during the synthesis of RNA. This metabolic error can interfere with RNA processing and protein synthesis (*Grem 2000*).

5-FU can be given parentally or orally as a prodrug, as capecitabine or UFT (a combination of tegafur and uracil) (*Hong et al. 2004; Nishida et al. 2003*). Less than 10% of 5-FU is protein bound in plasma. The dose varies from 300 to 1000 mg/m^2 daily, given in bolus injection schedules, or as a continuous infusion for several days. 5-FU is catabolised mainly in the liver to alfa-fluoro-ß-alanine (FBAL), urea and CO_2. FBAL is cleared in the urine. T½ is 10–15 minutes.

The maximum tolerated dose (MTD) and dose intensity (DI) (mg/m^2/week), for cytotoxic agents administered by bolus versus infusional schedules can be

considerable variable. The MTD and DI are influenced by infusion duration and interval between treatment cycles. The MTD and DI of 5-FU increase substantially with continuous infusional delivery (*Lokich and Anderson 1997*), as compared to bolus injections. The most important side-effects of 5-FU are bone marrow depression, mucositis and gastrointestinal toxicity as nausea, vomiting and diarrhea. The bone marrow toxicity is most dominant at bolus injections, and gastrointestinal toxicity dominates after continuous infusions. The biochemical basis for 5-FU toxicity has been attributed to impaired drug metabolism, resulting in markedly prolonged T½ and substantially reduced amount of drug catabolites. Clearance of 5-FU is lower given as bolus injection than by continuous infusion, supposedly due to saturation of DPD, the first step in 5-FU catabolism, and possibly by retention of 5-FU in tissues. The cardiotoxity and neurotoxicity of 5-FU is most prominent in high dose schedules, and is associated with high plasma concentrations of 5-FU and FBAL.

In our study, 5-Fluorouracil (Flurablastin® 50 mg/ml Pharmacia Sverige AB, Stockholm, Sweden), was obtained as a prepared solution in sterile isotonic sodium chloride in 2000 ml intravenous infusion bags. The pH of the intraperitoneal fluid was 9.1–9.2.

Leucovorin

Leucovorin (LV), the calciumfolin derivative of folic acid (citrovorum factor), increases the cytotoxic effect of 5-Fluorouracil by raising the intracellular concentration of 5,10-methyl-tetra-hydrofolate (mTHF). The mTHF forms a covalent complex with the active metabolite fluoro-deoxyuridine monophosphate (FdUMP) and thymidylate synthetase (TS), leading to a more long lasting blocking of TS.
Sequential administration of 5-FU and Leucovorin, gives simultaneous maximal concentrations of FdUMP and mTHF. The modulation of 5-FU by Leucovorin improves both response rate and survival in patients with advanced colorectal cancer, compared with 5-FU alone (*Thirion et al. 2004*). Very high doses of Leucovorin (400 mg/m^2) has not proven to be more efficient than low (20-50 mg/m^2) doses, but toxicity increases (*DeCaprio et al. 1991*).

Vasopressin

Vasopressin (lysin-8-vasopressin) is a vasoactive drug, that has been used clinically in a dose of 0.1–0.4 IE/min to treat bleeding varicose veins. The action of vasopressin is complex. The cutaneous, renal, coronary, muscular, and mesenteric arterial beds exhibit different reaction to vasopressin via specific V-2 and V-1 receptors (*Barthelmebs et al. 1996*). The dose 0.1–0.2 IU/min significantly constricts the splanchnic vessels, and decreases the portal venous pressure (*Ready et al. 1991*). We chose the lowest dose interval, 0.1 IE/min, as cardiovascular side effects are more prominent with rising doses.

Statistical analysis

Data are given as mean ± standard error of mean (SEM) in the animal studies or mean ± standard deviation (SD) in the clinical studies. The different groups were compared by means of Student's t-test for paired samples and ANOVA for group analyses. AUC was calculated according to the trapezoidal rule with Graph Pad Prism 3.0 (Graph Pad Software Inc. San Diego, CA, USA). All fitting calculations were done with the Origin 6.1 Graph and Data Analysis program (Origin Lab Corporation, Northampton, MA, USA) (paper II-III). Statistica (Statsoft Inc., Tulsa, OK, USA) was used for other calculations. A p value less than 0.05 was considered significant.

8. Results

Peritoneal blood flow

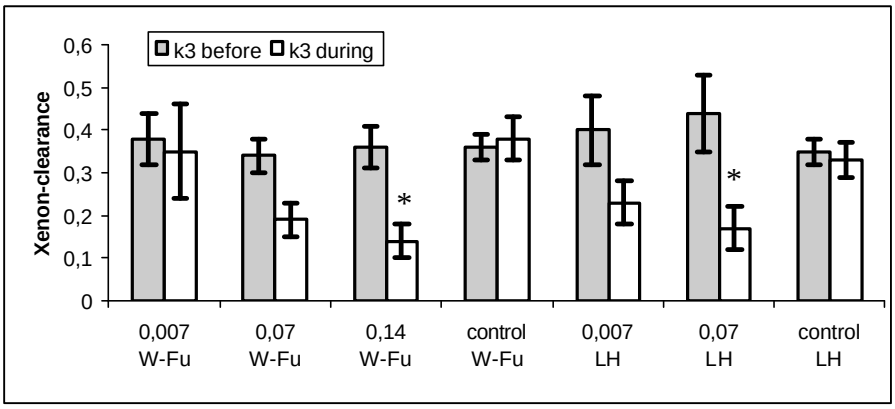
In this study the blood flow in the peritoneal cavity in two rat strains was estimated with the ^{133}Xe-clearance technique before and during intravenous vasopressin infusion analysed in two compartmental models.

^{133}Xe-clearance technique

The basal ^{133}Xe-clearance in all animal groups was 0.37±0.05 in the single compartment (k_3) of the mono-exponential equation, and 0.10±0.01in the slow compartment (k_2) of the bi-exponential equation with no difference between the groups. The single compartment (k_3) in the mono-exponential equation, and the slow compartment (k_2) of the bi-exponential equation gave reproducible information.

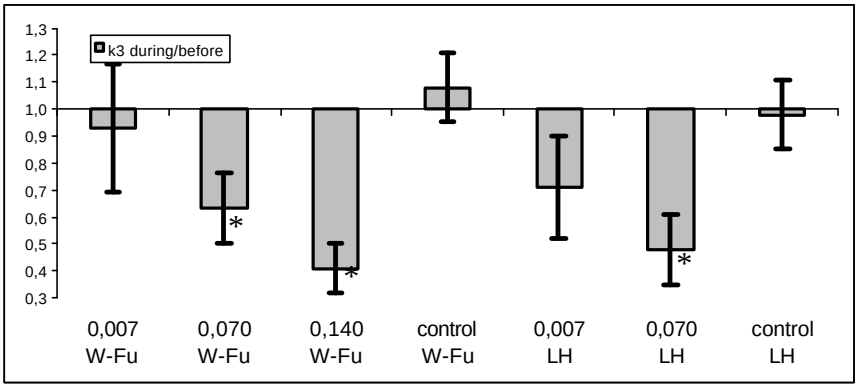
Vasopressin 0.007 IU/kg/min did not reduce the ^{133}Xe-clearance in any compartment model, neither in absolute, nor in relative values. Vasopressin 0.07 IU/kg/min reduced significantly the ^{133}Xe-clearance in relative values in the single compartment (k_3) and in the slow compartment (k_2) of the bi-exponential equation in both animal models. Vasopressin 0.14 IU/kg/min reduced significantly the ^{133}Xe-clearance in both absolute and relative values in W-Fu rats (Figure 9). Referring to the relative changes in the single compartment model, there was a significant decrease in ^{133}Xe-clearance of vasopressin 0.07 IU/kg/min in both rat models (Figure 10).

Figure 9 ^{133}Xe-clearance in absolute values in the single compartment (k_3) of the mono-exponential equation



Legends: Student's paired t-test during versus before. * = significant. k_3 before and during infusion of 0.007, 0.07 and 0.14 IU/kg/min vasopressin and in control animals. Results given as mean ± SEM.

Figure 10 ^{133}Xe-clearance in relative values in the single compartment (k_3) of the mono-exponential equation.

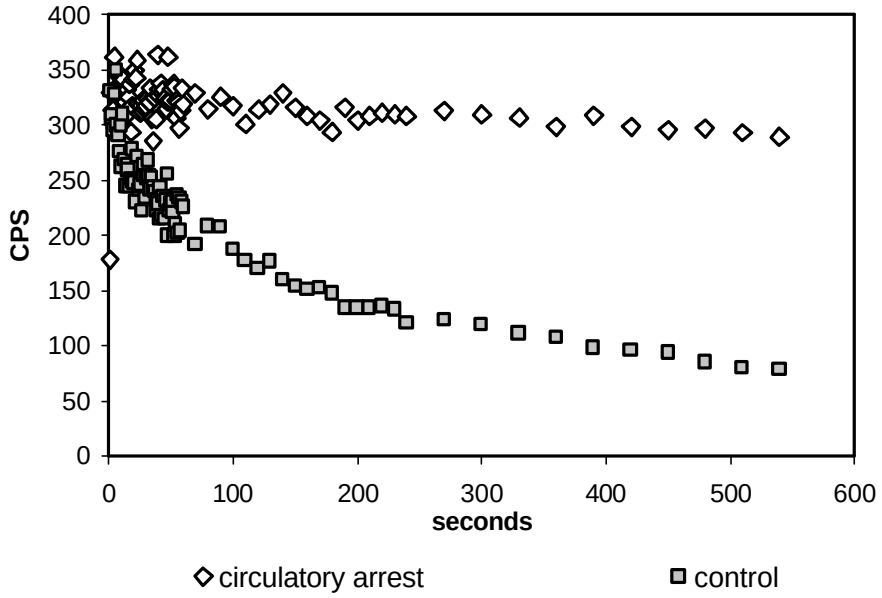


Legends: Student's paired t-test during versus before. * = significant . k_3 before and during infusion of 0.007, 0.07 and 0.14 IU/kg/min vasopressin and in control animals. Results given as mean ± SEM.

Vasopressin 0.007, 0.07 and 0.14 IU/kg/min did not affect ^{133}Xe-clearance in a reproducible way neither in absolute, nor in relative values in the fast compartment (k_1) of the bi-exponential equation (data not shown).

Using the gamma camera as a detector of radiation, the ^{133}Xe-clearance in the single compartment model (k_3) was 0.23 ± 0.026 and in the double compartment (k_1) and (k_2) 1.68 ± 0.27 and 0.071 ± 0.015. The animal with circulatory arrest showed a (k_3) of 0.014 and (k_1) and (k_2) of 0.045 and 0.0085, respectively. No dispersion outside the abdominal cavity was observed in the distribution images of the whole animal. Ten minutes after an intraperitoneally placed injection, more than 95% of the activity was gone. If the animal had circulatory arrest, more than 90% of the activity remained in the abdominal cavity after 10 minutes. Injection intentionally in the abdominal wall showed markedly retention after 10 minutes (Figure 11).

Figure 11 Gamma camera uptake of xenon washout in animal with intact circulation and with circulatory arrest.



Legends: CPS counts per second

Laser doppler flow

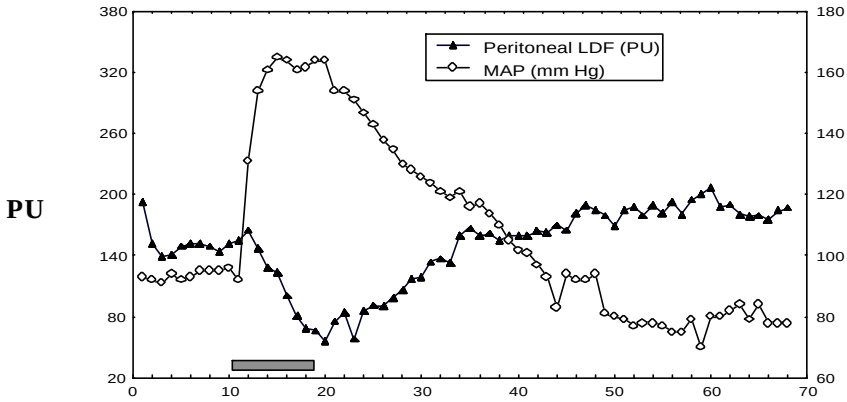
The Laser doppler flow showed the same pattern of decreased circulation during vasopressin infusion as the ^{133}Xe-clearance did. Vasopressin at 0.07 IU/kg/min induced a significant reduction in Laser doppler flow in both rat models in both absolute and relative figures (Table 3). The reduction was prompt and followed inversely the rise in mean arterial blood pressure (Figure 12).

Table 3 Absolute and relative LDF before and during infusion of vasopressin 0.07 IU/kg/min

Rat	N	PU BEFORE	PU DURING	P*	DURING /BEFORE	P **
W-FU	3	172±23	53±13	0.010	0.31±0.11	0.0019
LH	5	296±57	159±38	0.040	0.56±0.09	0.0039

Legends: Results given as mean ± SEM, *Student's paired t-test during versus before, **Student's paired t-test ratio during/before versus 1, PU = perfusion units.

Figure 12 Laser doppler flow in peritoneum and mean arterial blood pressure before and during infusion of vasopressin 0.07 IU/kg/min.

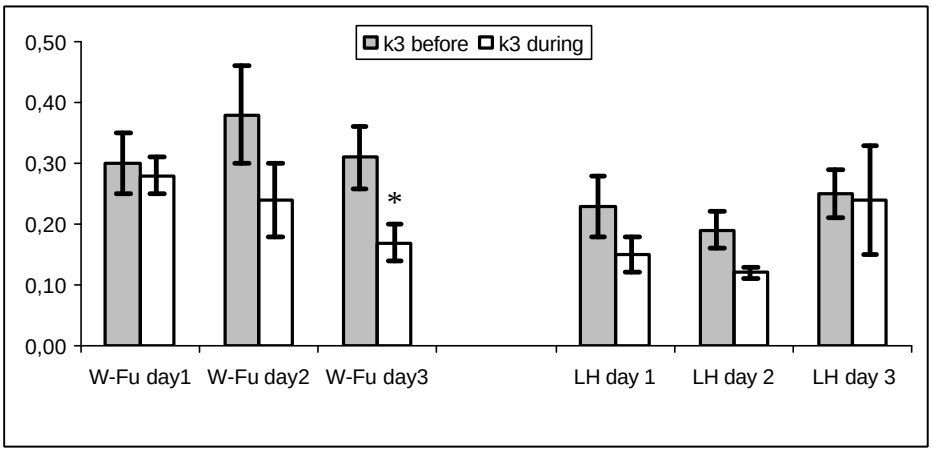


Legends: Filled rectangle indicates time of infusion of vasopressin, MAP = mean arterial blood pressure in mmHg, LDF = Laser doppler flow, PU= perfusion units.

Intraperitoneal 5-FU

There was a statistically significant reduction of the ^{133}Xe-clearance in absolute values during vasopressin administration in the W-Fu rats given intraperitoneal 5-FU ($p=0.027$). The relative values revealed a numerical decrease to 0.81±0.11 ($p=0.10$). A subgroup analysis identified a lesser reduction of the ^{133}Xe-clearance in W-Fu rats on day 1 and 2. On day 3 after intraperitoneal 5-FU the ^{133}Xe-clearance was significantly reduced (Figure 13). In LH rats given intraperitoneal 5-FU there was no reduction of the ^{133}Xe-clearance in neither absolute nor relative values. A borderline decrease was found on day 2 after the intraperitoneal administration of 5-FU. In relative values (0.68±0.11) this decrease was significant ($p=0.026$).

Figure 13 ^{133}Xe-clearance in the single compartment (k_3) in animals treated with intraperitoneal 5-FU.

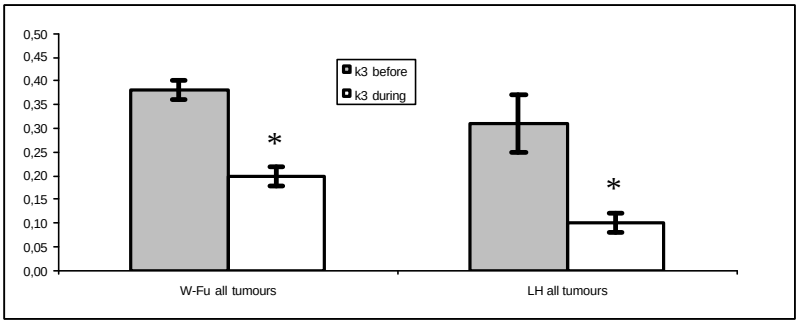


Legends: Student's paired t-test during versus before. * = significant . k_3 before and during infusion of 0.07 IU/kg/min VP .Results given as mean ± SEM.

Peritoneal carcinomatosis

In the tumour-bearing rats, the basal ^{133}Xe-clearance in W-Fu and LH rats (0.38±0.02 and 0.31±0.06, respectively) was of the same magnitude as in the sham group (0.34±0.04 and 0.35±0.06, respectively). The reduction in ^{133}Xe-clearance achieved with vasopressin was significant in both rat strains with small, medium or large tumour burden(Figure 14). The relative values of ^{133}Xe-clearance during versus before intravenous vasopressin was also significantly lowered in all rats with tumour.

Figure 14 ^{133}Xe-clearance in the single compartment (k_3) in animals with intraperitoneal carcinomatosis.



Legends: Student's paired t-test during versus before, * = significant, k_3 before and during infusion of 0.07 IU/kg/min VP and in control animals. Results given as mean ± SEM.

Clinical studies

Feasibility of intraperitoneal 5-FU treatment

The intraperitoneal treatment was easy to perform for the personal with few complicating events, and it was also readily accepted by the patients. The total duration of the treatment was most often less then two hours. One patient, with laparoscopically applied PAC, had leakage of the instillated fluid through the needle hole at the PAC site two hours after treatment. The PAC was adjusted as it was found that a channel was formed along the catheter. In two patients the PAC catheter had to be surgically corrected to obtain optimal distribution of the isotope within the peritoneal cavity. In four patients the PAC became infected and had to be removed, with no further IP treatment.

Effect of intraperitoneal 5-FU treatment

Table 4 Summary of survival, time to progression, tumour response and secondary chemotherapy.

	All n=68	**0 mg n=12**	**750mg n=18**	**1000mg n=12**	**1250mg n=12**	**1500mg n=14**
Survival *						
All N=68	8.0 (0.8-54.1)	4.4 (0.8-9.4)	7.6 (0.8-22.1)	8.7 (2.6-54.1)	14.7 (4.4-41.5)	9.1 (1.5-19.7)
+ LM n=23	6.3 (1.5-38.7)	3.5 (1.5-8.1)	4.8 (2.9-13.1)	7.1 (2.6-38.7)	12.8 (11.9-13.6)	6.5 (1.5-10.5)
– LM n=45	9.6 (0.8-54.1)	4.6 (0.8-9.4)	8.2 (0.8-22.1)	15.1 (6.2-54.1)	17.9 (4.4-41.5)	11.0 (3.9-19.7)
Time to progression *						
All n=68	4.4 (0.8-54.1)	3.8 (0.8-8.0)	5.5 (2.6-19.6)	3.6 (2.2-54.1)	8.2 (2.8-21.6)	4.1 (2.7-14.5)
Tumour response **						
SD***	27	NA	8	4	8	7
PR	2	NA	1	0	1	0
CR	1	NA	0	1	0	0

Legends: * Median months (range). ** Number of patients. *** SD = Stable disease evaluated at three months. – LM = no liver metastases; + LM = liver metastases. PR = partial response. CR = complete response. NA = not assessable.

The median survival of all 226 admitted patients was 6.8 months (0.1–114.1+). The 20 patients given systemic IV chemotherapy had a median survival of 4.3 months (0.8–8.9). The 81 patients given best supportive care, without any chemotherapy, had a median survival of 3.8 months (0.6–21.8). The median survival time of the 68 patients included in the intraperitoneal 5-FU study was 8.0 (0.8–54.1+) months.

The median survival in patients with liver metastases (n=23) was 6.3 months (1.5–38.7), and in patients without liver metastases 9.6 months (0.8–54.1+). The difference between these to groups was significant ($p<0.038$). Patients with peritoneal metastases did not have a significantly reduced median survival compared to those without peritoneal metastases, 7.4 months (0.8–54.1+) and 9.1 (0.8–40.1), respectively. One patient has an ongoing complete response (CR) (54.1+ months) and 2 patients experienced partial response (PR). Thirty patients achieved tumour control (CR+PR+SD) at three months.

After discontinuation, 54 patients were submitted to best supportive care (n=34) or to systemic chemotherapy with gemcitabine or 5-FU/Leucovorin (n=17 and n=3, respectively). Two patients are presently in treatment. The median survival after cessation of intraperitoneal treatment was 1.6 (0.1–17.4) months for patients who afterwards received best supportive care and 7.1 (0.8–41.0) months for patients given systemic chemotherapy. The overall survival of patients given secondary systemic chemotherapy or best supportive care was 10.2 (3.7–54.1+) months and 6.5 (0.8–38.8) months, respectively. Of patients given systemic chemotherapy and patients given best supportive care as secondary treatment, 23% and 21% had more than 12 months survival after inclusion in the intraperitoneal study. The difference in survival between patients given secondary systemic chemotherapy and BSC, could possibly be explained by the good performance index at cessation of treatment. The KI of patients given second line treatment was 85% (60–100), compared to KI for patients allocated to best supportive care 70% (50–100). There was no difference in weight reduction during treatment between the group given secondary systemic chemotherapy, median 95% (85–100) and BSC, median 97% (69–118).

Of the 18 patients who survived for at least 12 months, ten tumours were morphologically and eight tumours cytologically diagnosed. The survival of all patients with morphologically diagnosed tumours was 7.6 (0.8–54.1+) months and cytologically diagnosed 8.1 (0.8–22.1) months ($p>0.05$). The range of 5-FU dose per two-day treatment administered in our study was 2736 to 4882 mg, resulting in a dose/week of 912–1627 mg.

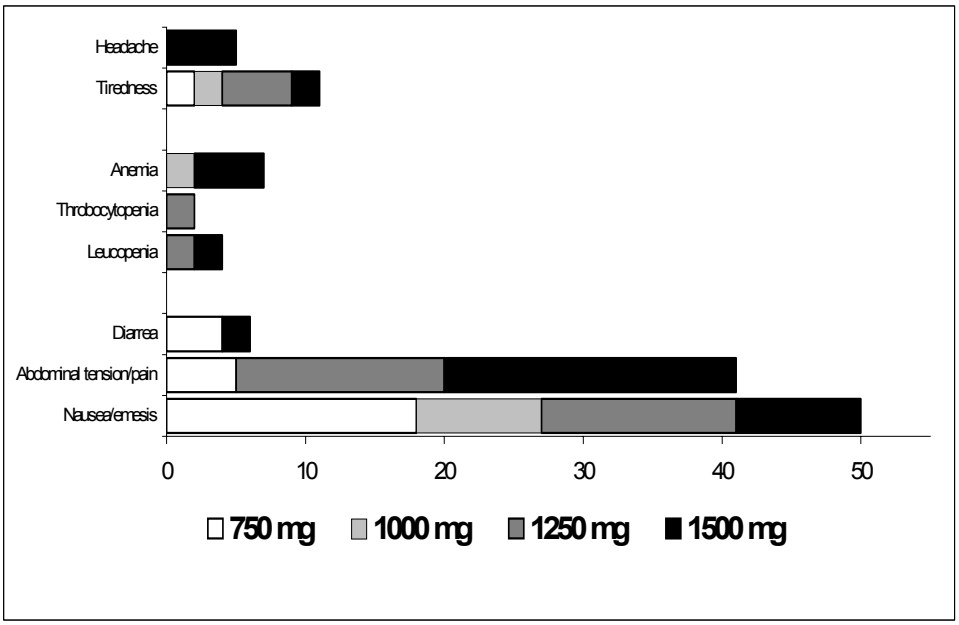
Safety of intraperitoneal 5-FU treatment

The treatment was well tolerated, with no major complications or side effects. There was no signs of bone marrow depression, mucositis or treatment-related episodes of sepsis or mortality. No patient was excluded due to toxic side effects. Abdominal pain and symptomatic distension was more frequent in doses exceeding 1000 mg/m^2. Nausea and emesis was more frequent in patients receiving intravenous vasopressin and showed no tendency to increase as doses were raised (Figure 15).

Three patients receiving 1500 mg/m^2 5-FU experienced angina pectoris (WHO toxicity Grade 3) with elevated transaminases and Troponin I several hours after

the IP treatment. This side effect has earlier been reported with continuous intravenous infusions of 5-FU. It is probably due to saturation of the rate limiting step of 5-FU catabolism, the DPD activity, resulting in high plasma levels of 5-FU.

Figure 15 Worst WHO toxicity Grade I and II across all cycles of therapy



Legends: Percent of treated patients (n=56) experience the event at least once. All events of nausea/emesis in patients given 750 mg/m^2 occurred when vasopressin was given.

Fifteen patients underwent laparotomy during treatment. Three patients presented with signs of bowel obstruction and twelve patients with duodenal compression that required a gastric bypass. In five cases moderate adhesions were found. Two patients with peritoneal metastases found at the primary operation, were operated after 2.1 and 3.3 months IP 5-FU treatment, and they had no macroscopic peritoneal metastases at the second operation. One patient, who underwent secondary laparotomy after 21.1 months had developed peritoneal metastases during treatment. Of the five patients found to have a moderate adhesions at the second operation, two had incomplete scintigrafic distribution 1.3–2.1 months prior the operation. The other three, with duodenal compression as indication for surgery, had good distribution of the isotope in the abdominal cavity 2.2–7.7 months prior the second operation.

The median treatment duration was 3.9 (0.1– 26.9) months in all patients. In patients that after intraperitoneal treatment received systemic chemotherapy, or were given BSC, the duration of the intraperitoneal treatment was 2.6 (0.1–15.5) and 6.4 (0.1–26.9) months, respectively. The indication to stop IP 5-FU in the

patients given chemotherapy as second-line treatment, was progression on CT in 67% (14/21), clinical progression (3/21), patient withdrawal (2/21) and choice of the referring hospital (2/21). The indication to stop IP 5-FU in the group given BSC after IP 5-FU treatment, was progression on CT in 21% (10/47), clinical progression (18/47), patient withdrawal (3/47) and choice of the referring hospital (2/47).

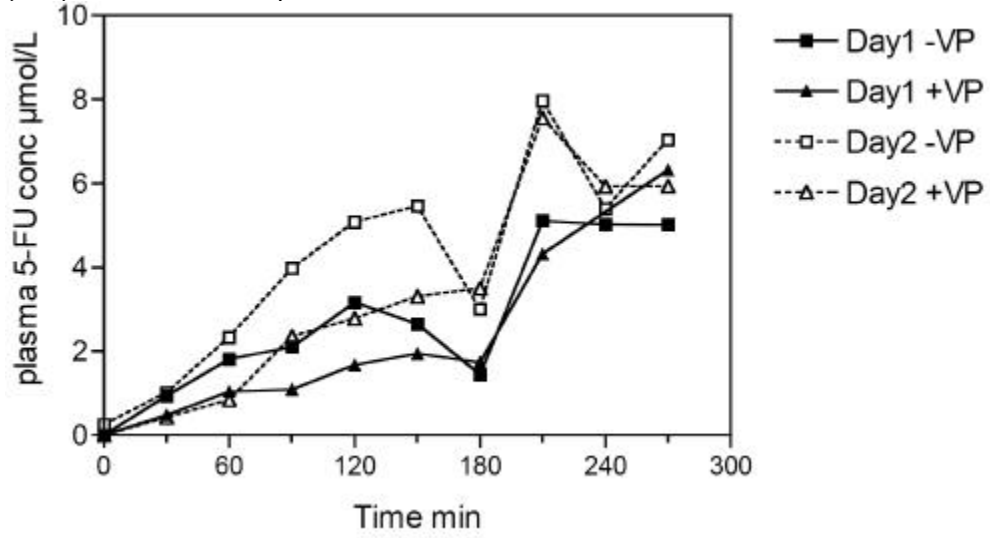
Pharmacokinetic study

The pharmacokinetic study was done on patients receiving 750 mg/m^2 5-FU. Both 5-FU Cmax and AUC was lower when intravenous vasopressin was given. There was a significant reduction of Cmax on day 2 ($p<0.05$) when intravenous vasopressin was given.

We tried to handle samples in a standardised way after they were drawn until placed in the freezer. Paired peritoneal and plasma samples were drawn, and immediately centrifuged and frozen, or left in room temperature for two hours before handling, and there was no difference in measured 5-FU concentrations between the two handling procedures.

When intravenous vasopressin was given day 1 or 2, both 5-FU Cmax and AUC were lower than the days when no vasopressin was given. Only for Cmax day 2 was the reduction significant ($p<0.05$). For days 1 and 2 when vasopressin was given, 5-FU plasma Cmax was 2.34 ± 1.52 and 3.46 ± 2.46 µmol/l, and AUC was 70 ± 44 and 118 ± 69 µmol/l x hr, respectively. Without intravenous vasopressin day 1 or 2, 5-FU plasma Cmax was 4.25 ± 3.17 and 6.11 ± 5.37 µmol/l, and AUC was 127 ± 95 and 118 ± 69 µmol/l x hr, respectively.

Figure 16 Plasma 5-FU concentration Day 1 and Day 2, with (+VP) and without (-VP) intravenous vasopressin infusion.



9. Discussion

Peritoneal blood flow

In our animal experiments, we explored a new way to measure peritoneal blood flow changes with the ^{133}Xe-clearance technique. We found that the method gave reproducible results of peritoneal blood flow changes achieved by intravenous infusion of the vasoconstrictor vasopressin. The effect of intraperitoneal installed 5-FU temporarily hampered the reduction of peritoneal blood flow by intravenous vasopressin, while peritoneal carcinomatosis neither affected basal, nor vasopressin-altered peritoneal blood flow.

The injection is intended to disperse the Xenon gas in the whole abdominal cavity. If the isotope is deposited in fatty tissue, either in the abdominal wall or in the abdominal cavity, the washout curve will hardly exhibit a first order compartment (k_1), but only a slow second order compartment (k_2). If the isotope is deposited directly into a vessel the disappearance of the xenon is extremely rapid, as it is immediately transported out of range of the collimator.

Much effort was spent to reduce the bias of temperature, circulatory blood volume, feeding and anaesthesia. Animals that did not maintain a temperature over 36.0 °C were excluded. During the experiments, 2–3 ml isotonic saline was injected intramuscularly every hour to ensure adequate circulating blood volume. All animals were on a standard pellet diet and the experiments were performed with animals from the different groups allocated randomly during the day.

^{133}Xe-clearance technique

Compartmental analysis of the ^{133}Xe-clearance curve, is an application of the general use of inert gas washout curves to measure organ blood flow, or more specifically, tissue perfusion. We applied two models to analyse the curves, the bi-exponential and the mono-exponential. Our intention was to use the most reproducible, yet straightforward and simple analysis.

Well-perfused areas absorb ^{133}Xe by passive diffusion at the same rate as badly perfused areas. In areas with low perfusion, the elimination of the isotope is delayed. The ischemic or less perfused tissue serves as a reservoir for the isotope, because it has to diffuse back into well-perfused tissue to be eliminated. In the absence of circulation, the injected ^{133}Xe cannot be transported away from the abdominal cavity. The absence of measurable ^{133}Xe washout in rats with circulatory arrest, demonstrates the necessity of blood perfusion of the area for washout to take place.

After repeated ^{133}Xe-injection, small residues could remain in the tissue due to previous injections. However this ^{133}Xe represent only a very low activity, much less than 100 CPS, and thus minimally affects the k-value (the declination of the

slope). The time elapse between the measurements could be 30–40 minutes allowing most of the activity to be washed away.

The single compartment model (k_3) as presented in paper II, gave congruent and reproducible information of the washout function, and is derived from the curve from 10 to 100 seconds. It has the benefit that the curve does not have to be adjusted to the first point from which k_1 is determined, or the very low counts in the last part of the curve that decides k_2. The slow compartment (k_2) of the bi-exponential equation displayed a reduction in ^{133}Xe-clearance with vasopressin in agreement with the single compartment (k_3), in the mono-exponential equation. The fast compartment (k_1) of the bi-exponential equation, gave much less reproducible information. The k_1 can be considerably affected by the injection technique of the isotope and the k_2 can be disturbed by retained residual activity in peri-peritoneal fatty tissue.

Using the slow compartment also requires acquisition of counts over a considerable time, in this experiment during 10 minutes. Knowing that blood flow changes are dynamic, and that factors of bias will add over time, it is supposedly better not to rely on sampling of data over a long period of time. The single compartment model, offers the possibility of shorter data acquisition periods.

The ^{133}Xe-clearance method has also been validated against microspheres, electromagnetic flowmetry and quantitative perfusion fluorometry for liver blood flow with good correlation (*DeMar et al 1989; Thom et al. 1988*). The reproducibility of measuring tumour blood flow with ^{133}Xe-clearance has been addressed (*Bolmsjö et al. 1983; Naredi et al. 1992*). In our study, the reliability of the ^{133}Xe-clearance technique as method for peritoneal blood flow estimation, was supported by invasive Laser doppler flowmetry. The registered reduction of blood flow with 0.07 IU/kg/min vasopressin was of the same magnitude with both methods.

Intraperitoneal 5-FU

^{133}Xe-clearance was measured one, two and three days after intraperitoneal 5-FU was given. We did not analyse day zero because our intention was to analyse the effect of consecutive treatments, not to explore the immediate effect on the peritoneal blood flow. In W-Fu rats there was no effect on the basal ^{133}Xe-clearance values, but in LH rats these values were reduced to approximately 2/3 from 0.35 to 0.22. A speculative explanation for the difference in reduction of basal ^{133}Xe-clearance between W-Fu and LH rats could be a different sensitivity to 5-FU of vessels in the peritoneal cavity.

In the animal experiment with intraperitoneal 5-FU, there was no difference in MAP during vasopressin infusion in the 5-FU treated W-Fu and LH rats. This indicates that there is no general circulatory vasoconstrictive effect of 5-FU in either rat strain. It would not be controversial to believe that absence of systemic

reaction also applies to the peritoneal microcirculation. IP 5-FU appears to have a local and not a systemic effect. This local effect could also affect the peritoneal absorption of 5-FU. In the clinical study, an increased absorption of IP 5-FU on the second day of treatment is suggested. In both rat-strains intravenous vasopressin failed to demonstrate a reduction of the ^{133}Xe-clearance one day after intraperitoneal administration of 5-FU. Our interpretation of the results is that intraperitoneal 5-FU, might initially affect the peritoneal blood flow, thus influencing the kinetics of the drug.

Peritoneal carcinomatosis

Malignant cells spread in the abdominal cavity directly or through lymph and blood. The cells adhere to the tissue by blood clots and fibrin and are exposed to growth factors brought there by the peritoneal capillaries. The growth of the peritoneal micrometastases is most pronounced in areas of wound healing, sutures and on the raw peritoneal surface (*Sugarbaker 1996*). The development of peritoneal carcinomatosis is considered a terminal stage of tumour progression.

In our experimental set-up, there was no difference in neither basal nor vasopressin affected ^{133}Xe-clearance in control rats and rats with intraperitoneal tumour burden. Not even extensive carcinomatosis seemed to effect clearance in any direction. The remaining peritoneal lining left in rats even with a large tumour burden, seems to be large enough to allow a basal peritoneal blood flow, still with the ability to react to vasoconstrictors. It appears, at least theoretically, applicable to reduce the peritoneal blood flow with vasoconstrictors, thus reducing the absorption and transport via the capillaries and portal system.

Influence of vasopressin

Drugs administered intraperitoneally are cleared either by the capillaries leading to the portal venous system, or by intraabdominal lymphatics. It has been proposed that clearance of small solutes from the peritoneal cavity in the model of dialysis is limited more by low effective blood flow than by low permeability of the peritoneal membrane (*Ronco et al. 1996*). Decreased splanchnic blood flow, achieved with vasopressin, may reduce the uptake of cytotoxic drugs from the cavity and hereby raise the dose intensity in the abdominal cavity and redirect the clearance to the intraabdominal lymphatic tissues without increasing systemic toxicity.

In an experiment on dogs, 0.008 IU/kg/min vasopressin given as an intra-aortic infusion, reduced the superior mesenteric artery blood flow to 33% and Xenon-washout registered from jejunal submucosa was reduced to 50% of baseline value (*Wilson et al. 1976*). In our work, 0.07 IU/kg/min vasopressin IV decreased the ^{133}Xe-clearance to 37% of baseline value, as an expression of reduced peritoneal blood flow. The vasoconstriction was sustained during the whole experiment,

recorded by a consistent level of MAP, as well as by the Laser doppler flowmetry experiment.

A vasoconstrictive agent that decreases the capillary blood flow and perfused capillary area, could increase the total tissue penetration and maybe selectively enhance tumour blood flow and drug delivery. This has been demonstrated in liver-tumour blood flow studies where angiotensin, epinephrine and vasopressin increased tumour blood flow (*Lindner et al. 2004; Sasaki et al. 1985; Ackerman et al. 1989; Hemingway et al. 1991*). Due to the low tone in tumour blood vessels, changes in blood flow are achieved by alterations in resistance of the host vascular bed and a changed perfusion gradient due to variations in systemic blood vessels (*Naredi and Hafström 1992*). A beneficial effect of vasopressin on 5-FU uptake in tumours is supported by the observation that vasopressin given in the hepatic artery has been shown to increase uptake of 5-FU in liver metastases (*Dietz et al. 1998*).

Clinical studies

Intraperitoneal chemotherapy

This study shows that intraperitoneal 5-FU administration to patients with non-resectable pancreas cancer has anti-tumour effect with minor toxicity. The intraperitoneal route offers pharmacokinetic advantages which have been very little studied in pancreas cancer.

The abdominal cavity is a compartment from which drugs can be introduced in the body. The exposure of the tumour to a drug in the peritoneal fluid is determined by pharmacokinetic factors, such as drug concentration, rate of absorption and fluid composition, as well as local factors such as inflammation of the peritoneal lining and adhesions between structures. Drugs are transported from the peritoneal cavity by intra-abdominal lymphatics and by capillaries leading to the portal venous system and the liver. Due to the much higher blood flow in the portal route this way of clearance dominates (*Speyer et al. 1981; Cunliffe and Sugarbaker 1989*). The ratio of total drug exposure (area under the concentration x time curve (AUC)) for the peritoneal cavity relatively to that for plasma, is determined by the clearance of the drug from the peritoneal cavity and from the systemic circulation.

Intraperitoneal administration of chemotherapeutic drugs as 5-FU, cisplatin, mitomycin C and pacitaxel has a favourable peritoneal to plasma drug ratio, exposing the abdominal cavity and lymphatics to a high local drug concentration. Mitomycin C has a high local toxicity, making it necessary to reduce the intraperitoneal dose, even though it is rapidly cleared from the systemic circulation. Pacitaxel, although the regional advantage in terms of peritoneal AUC versus plasma AUC is 1000 to 1 (*Markman et al. 1993*), has slow clearance from the abdominal cavity, so the systemic effect is supposedly low. 5-FU and cisplatin have the opportunity to act systemically after absorption in capillaries and

lymphatics. 5-FU has very low local toxicity, render it an ideal drug to be administered in the peritoneal cavity. The intraperitoneal dose of 5-FU can be increased to the point where the amount of drug entering the systemic circulation is equivalent to the maximum dose that could be administered by the intravenous route.

Intraperitoneal chemotherapy, has the greatest advantage in treating small residue disease after debulking surgery and peritoneal carcinomatosis. Peritoneal metastases may represent the single most important factor in the treatment failure of pancreas cancer (*Muchmore et al. 1996*). In palliative treatment of pancreas cancer, reduction of the loco-regional and lymphatic spread, would presumably slow down the progress of the disease and prolong survival.

Feasibility
The intraperitoneal treatment was simple to handle, and has the potential to be carried out at home, with the aid of a nurse. Placing the PAC on the lower rib cage made it easy to localise the port and insert the needle, thus achieving most possible comfort for the patient. The low frequency of port site infections was encouraging. The installation of 2000 ml room tempered isotonic sodium chloride was well tolerated. There was some considerations giving the same volume to large and small patients. Adjusting the volume to the body surface area could be a proper way to reduce the abdominal distension for patients with a small stature, and ensure an adequate exposure of the peritoneum in patients with large body surface. A volume of 1000 ml/m^2 could be reasonable, giving a range from 1500 to 2200 ml in most patients. This would, except for concentration, not significantly alter the physical properties of the fluid. The pH of the solution is 9.1–9.2, regardless of 5-FU concentration. With the Technetium-99 scintigraphy, an excellent distribution within the peritoneal cavity could be confirmed in almost all patients. There were few symptoms related to adhesions in the abdominal cavity during the intraperitoneal treatment.

Safety
The treatment was well tolerated with no WHO Grade 3 or 4 toxicity with 5-FU doses up to 1250 mg/m^2. At 1500 mg/m^2 three patients showed signs of cardiac ischemia on the second day of treatment, and in one patient activity-associated mild chest pain was noted for 3–5 days after treatment. Angina is a known adverse event from 5-FU treatment (*Wacker et al. 2003*). This has been found with high plasma levels of 5-FU and FBAL. It might depend on saturation of the first rate limiting step, the DPD activity, of the 5-FU catabolism. A close observation of patients receiving the first intraperitoneal 5-FU treatment, should be sufficient to identify patients at risk.

No patient had haematological toxicity that required cessation of therapy. The maximal plasma concentration of 5-FU (Cmax) in the group given 750 mg/m^2 was 6.1±5.4 µmol/L. Cmax in one patient receiving 1500 mg/m^2 was 17 µmol/L the second day of treatment. These Cmax are low compared to Cmax reached in

intravenous bolus injections of 5-FU, where plasma peak concentrations of several hundred µmol/L were recorded (*Grem 2000*). In infusional schedules, with the duration of infusion from several hours to days, the Cmax is 10–20 times lower than with bolus injection. The myelosuppression in 5-FU treatment is related to the maximal plasma concentrations. The very low toxicity profile of the intraperitoneal 5-FU treatment, is likely due to the low Cmax. In our study, 1500 mg/m^2 intraperitoneal 5-FU given two consecutive days every 21 days, aimed at a dose of 1700 mg/week. This is higher than proposed in studies of intraperitoneal 5-FU with cycles of 7 days, in which 1200 mg/week was judged reasonable as a single drug (*Budd et al. 1986*), or 3900 mg/4 weeks in combination with cisplatin (*Schilsky et al. 1990*). A cycle of 21 days, as in our study, lets the bone marrow recover between treatments, and is probably better to avoid myelosuppression. The toxicity of the intraperitoneal 5-FU treatment is considerably lower than encountered in treatment with gemcitabine, where vomiting (10–27%), alopecia (50%), oral ulceration (14%) and leucopenia (14–26%) is more frequent (*Palmer et al. 1994; Ko et al. 2004; Burris et al. 1997*). Combinations of gemcitabine and other drugs, i.e. oxaliplatin, tipifarnib and pemetrexed recently failed to show significant survival benefit but added further toxicity (*Louvet et al. 2004; Richards et al. 2004; van Cutseem et al. 2004*). Taken together, the low toxicity profile identifies the intraperitoneal 5-FU schedule as a suitable palliative treatment for patients with non-resectable pancreas cancer, even for those in the lower rank of performance status.

Tumour response

Generally patients with advanced disease present with liver or peritoneal metastases in 23–40% and 25–35%, respectively (*Brennan et al. 1993*). In our study, liver or peritoneal metastases was found in 33% and 29%, much alike other patients with advanced disease. There was a significant difference in median survival in patients with or without liver metastases (6.3 and 9.6 months). Median survival for patients with metastatic pancreas cancer is low, and in recent studies giving combination chemotherapy, median survival is 5.6–7.3 months (*Funakoshi et al. 2004; Li and Chao 2004*). In our study, the median survival for patients with liver metastases, was the same as for patients receiving much more toxic chemotherapeutic regiments. Patients with peritoneal metastases did not have a significantly reduced median survival time compared to those without peritoneal metastases (7.4 and 9.1 months). Intraperitoneal 5-FU is apparently effective in reducing the malignant potential of peritoneal metastases. This speaks in favour of intraperitoneal 5-FU treatment for patients with peritoneal metastases.

The decision to stop intraperitoneal 5-FU treatment in the group thereafter given BSC was based on clinical progression, rather than progression on CT (38% vs. 21%). For the group given systemic therapy as second-line treatment, the relations were the reverse, where clinical progress stopped treatment in 14% and CT progress stopped treatment in 67%.

The CT scan interpretation of a stroma rich tumour, as pancreas cancer, is notoriously difficult. The fibrosis and necrosis within and around the tumour is

difficult to distinguish from previous viable tumour. Correlation between the CT estimates of tumour diameter and actual tissue measurement is good for tumours greater than 2 and less than 5 cm in diameter (*Sohaib et al. 2000*). Smaller tumours tends to be overestimated and large or diffuse tumours underestimated, because of their form, cystic and necrotic parts or ill defined borders. The RECIST (Response evaluation criteria in solid tumours) criteria (sum of longest diameter) has been advocated instead of the WHO index (sum of products), to overcome the difficulties of tumour size estimation (*Park et al. 2003; Trillet-Lenoir et al. 2002*). In this study a retrospective estimation according to the RECIST criteria of all thin sliced helical CT scans from patients in the 1250 and 1500 mg/m^2 groups was done. We found no significant difference in tumour response between the RECIST and the WHO index (data not shown).

In the present study CT tumour response was judged according to the WHO criteria. The CT response rate of 4.4% in all treated patients is low and not obviously correlated with survival time. In the group treated with 1250 mg/m^2 5-FU, the response rate was 0% but the median survival time 14.7 months. This group had the lowest median age and also least frequent liver and peritoneal metastases, which partly explains the long survival time. The patient with CR and the two patients with PR had initially SD for 18.1, 3.0 and 4.5 months, respectively, where after tumour regression was observed. This difficulty in evaluating single CT scans for tumour response, could warrant for certain care in terminating the treatment, and in the absence of toxicity and clinical deterioration, not hasten to second-line treatment or even abort. Other means of defining endpoints of treatment are discussed below.

Selection of patients

A selection of patients with a KI of 70 or more does not differ from inclusion criteria in other studies of systemic treatments. The referral of 81 patients after preoperative evaluation to best supportive care or systemic chemotherapy, was due to patients being judged non-resectable on CT or MRI or that they did not tolerate an operation. Ninety-five percent were not given systemic therapy, thus representing a group of patients that supposedly would have been given best supportive care instead of being included in other studies.

Twenty patients who failed surgery and did not meet the inclusion criteria were referred to the department of oncology (Figure 5). Sixteen of these patients and four of the patients referred to the department of oncology after preoperative evaluation, were given primary systemic chemotherapy. This group had a median survival of 4.3 months (0.8–8.9). The patients given primary best supportive care (n= 81) had a median survival of 3.8 months (0.6–21.8). Of the 68 patients included in our study, six patients did not even start treatment due to rapid progression of the disease. Taken together, the group of included patients probably is representative for patients with advanced disease, and the survival time is comparable to what is attained in systemic chemotherapy treatments.

Pharmacokinetics

The pharmacokinetic analysis of Cmax and AUC focused on the effect of vasopressin and the day of 5-FU administration.

The plasma AUC and Cmax are highly variable depending on the schedule of 5-FU administration. A bolus injection within 3–5 minutes of 5-FU 370 mg/m^2, results in an AUC of 105 µmol/L x hr and a Cmax of 370 µmol/L, while the same dose in an one hour infusion resulted in an AUC of 25 µmol/L x hr and a Cmax of 25 µmol/L (*Bocci et al. 2000; Grem et al. 2001*). The intraperitoneal route resembles a continuous infusion more than a bolus injection. Continuous infusion of 5-FU in colorectal cancer has shown a slight superiority over bolus injections during 3–15 minutes regarding haematological toxicity and tumour response (*Meta-analysis Group in Cancer 1998*; *Glimelius et al. 1998*). In our study, the plasma 5-FU AUC during three hours from start of 5-FU instillation, ranges from 70 ± 44 to 194 ± 167 µmol/L x hr and Cmax from 2.3 ± 1.5 to 6.1 ± 5.4 µmol/L. The pharmacokinetic study was performed on patients receiving 750 mg/m^2 5-FU. In one patient receiving 1500 mg/m^2 the peritoneal and plasma 5-FU concentrations were analysed for the purpose of controlling the sampling handling procedure. Cmax was 10 and 17 µmol/l day 1 and day 2, respectively. This observation suggests that intraperitoneal 5-FU administered in these doses, reaches plasma concentrations as if it was given as an intravenous continuous infusion, and probably has the ability to exercise antitumour activity through the capillary bed.

There was a decrease of Cmax when adding vasopressin, and the decrease was significant on day 2 of treatment. No difference was found in 5-FU Cmax with or without vasopressin on day 1. Vasopressin had no significant effect on plasma 5-FU AUC either day. Interestingly, the day of administration changed the plasma 5-FU AUC, with a numerical, but not significant, increase of AUC on day 2 both with and without vasopressin. The difference in Cmax and AUC between day 1 and day 2 might depend on local factors due to the 5-FU affecting the peritoneum or a saturation of 5-FU catabolism via the DPD pathway, resulting in a higher Cmax of 5-FU day 2. If only the latter was a factor of concern, there would be no difference in 5-FU Cmax on day 2 with or without vasopressin. As this is not the case, the slightly higher 5-FU Cmax in plasma on the second day of treatment cannot be solely attributable to DPD saturation.

In peritoneal dialysis, the phenomena of hyper-permeability of the peritoneal membrane, has been observed during acute peritonitis, a mechanism possibly mediated by a vasodilatation caused by prostaglandins (*Maher et al. 1980*). A hyper-permeable membrane, would allow a faster diffusion across the interstitium to the capillaries and the systemic circulation, eventually raising the plasma concentration. A hypo-permeable membrane could be the result of a chronic inflammatory state with sclerosis and thickening of the membrane, thus reducing the systemic effects but also the penetration of the drug into the tissue. Peritonitis appears not to have effect on the structure and function of the lymphatic lacunae

(*Mactier 1988*). Giving intraperitoneal 5-FU during 1–2 days in three weeks interval is supposedly better than giving it for several consecutive days. The effect on the peritoneal microcirculation will be lower, avoiding the increased absorption as it increases the plasma concentration.

After 180 minutes, the 5-FU concentration in plasma increased markedly (Figure 16). A redistribution to other intraabdominal compartments, could explain this increased systemic 5-FU uptake as patients were permitted to move more freely after treatment. There are fewer observations after 210 minutes, as patients wanted to leave the outpatient clinic as soon as possible. In our study we found measurable plasma 5-FU concentrations during at least 390 minutes but not at 24 hours. The rate of lymphatic reabsorption may depend of the patients posture (higher in supine position) and intraabdominal pressure. In rats, true lymphatic absorption occurs primarily by lymphatic lacunae in the diaphragm (60%), and to a lesser extent by visceral lymphatics (30%). Parietal lymphatics stands for only 10% of the reabsorption. In our study, the marked raise in plasma 5-FU concentration after 180 minutes, is not explained by an increase in lymphatic absorption. Shifting to a more upright position would rather reduce the lymphatic reabsorption. What is probably more important by shifting position is that such exercise allows other areas of the peritoneal surface to be exposed to 5-FU.

Analysis of the peritoneal fluid 5-FU concentration after intraperitoneal administration, showed a first-order elimination with a half-life of 1.6 hours and with 82% of administered drug absorbed in 4 hours (*Speyer et al. 1981*). The lymphatic peak concentration of 5-FU in pigs treated with intraperitoneal 5-FU occurred after 30 minutes, and decreased thereafter. The lymphatic to plasma ratio was 5.7, when measured in the thoracic duct close to the diaphragm. At that level, the lymphatic fluid drained from the abdominal cavity, is presumably diluted by lymphatic fluid drained from other parts of the body. The intraperitoneal concentration of 5-FU was 38 times higher than the lymphatic concentration, suggesting that the abdominal lymphatic system closer to the peritoneal cavity, is more intensely exposed to the drug (*Lindner et al. 1996).* In our study, we had difficulties obtaining sufficient amount of peritoneal fluid for analysis due to the small size catheter. A shift to a larger catheter (i.e. Tenckhoff), was not done, as the PAC was well tolerated by the patients.

Carrier solution
There are various limitations of the intraperitoneal route, of which uneven distribution over the peritoneal surface within the abdomen due to adhesions after surgery or an insufficient volume of fluid leads to inadequate drug distribution. As mentioned above, the stationary position during instillation could also be a factor which influence drug uptake.

The impact of the carrier solution on the pharmacokinetic of intraperitoneal 5-FU in rats has been analysed. 5-FU left the peritoneal cavity independently of the type of carrier, but hypotonic and isotonic sodium chloride solutions were more rapidly

cleared than hypertonic solution and high molecular weight solutions (*Pestieau et al. 2001*). A high molecular carrier solution, as the iso-osmolar glucose polymer-based dialysate solution icodextrin, can prolong the availability of the chemotherapeutic drug at the peritoneal surfaces, by maintaining a large volume for an extended time (*Hosie et al. 2003*). In our study we used isotonic saline to maintain a high intraperitoneal volume for 4–6 hours when most of the 5-FU is cleared from the peritoneal cavity.

When the drug is transported into the tissue by diffusion, large volumes of intraperitoneal fluid, in most patients 2000 ml must be employed, to ensure that the drug actually reaches all parts of the peritoneal lining (*Dunnick et al. 1979; Keshaviah et al. 1994*). Adhesions, post surgical scarring and the patients position and movements during treatment present additional problems, in exposure of serosal surfaces. Using a large volume isotonic carrier solution, the whole abdominal cavity is ensured homogenous distribution, and as the absorption of the fluid is 1.1–1.4 ml/min in man, this leads close to total absorption of the intraperitoneal volume in 24 hrs (*Hosie et al. 2001; Heimbürger et al. 1995*).

Tissue penetration

The tissue penetration of a drug is a function of the tissue structure and the drug properties. In pancreas tumour the blood flow varies in different parts. There is often a hypervascular peripheral region with active neo-vascularisation and an avascular or even necrotic central portion. The interstitial pressure in tumours is elevated, counteracting the movement of drug diffusion into the tissues (*Curti et al. 1993*). The depth of penetration of intraperitoneally administered cytostatics into the tumour is limited, ranging from several cell layer to a few millimetres from the surface (*Ozols et al. 1979; Los et al. 1989*). 5-FU was in a rat model shown to have an extensive and non-linear metabolism, reaching 50% of surface concentration at a depth of 50–300 µm tissue in low concentration solutions. The depth was extended to 650 µm by using high concentration solutions to saturate the drug metabolism in the tissue (*Collins et al. 1982*). The intraperitoneal route of administration with high local concentration of 5-FU, reaches the tumour mass probably both by diffusion, and through absorption and capillary perfusion of the vascularized tumour mass.

Bulky disease, requiring deep tissue penetration, is a clear limitation of the intraperitoneal modality in a curatively intended treatment. Any strategy employing intraperitoneal therapy with a curative intention, requires small-volume disease. Intraperitoneal administration of chemotherapeutic drugs against intraabdominal malignancies was successful after surgically debulking and reducing the tumour to microscopic masses, giving up to 67% documented response rate at second look in cisplatin-sensitive patients (*Braly et al. 1995; Howell et al. 1987*). In rat models, intraoperative intraperitoneal 5-FU significantly reduced the incidence of port site tumour implantation (*Eshragi et al. 1999*) and significantly decreased peritoneal carcinomatosis (*Ridwelski et al. 2002*).

In our study there was no difference in survival time between patients with peritoneal metastases and those without, implying that the intraperitoneal 5-FU treatment had anti-tumour activity in the abdominal cavity, and reduced the progress of peritoneal metastases. Two patients, with peritoneal metastases at the primary operation, were reoperated due to bowel obstruction, and was found to have macroscopic complete remission of the peritoneal metastases. Therefore it is plausible, that intraperitoneal 5-FU should not be regarded as a curative treatment for non-resectable pancreas cancer, but a palliative treatment with the option of reducing the development of peritoneal metastases, and probably also by acting systemically.

Expanding the hypothesis of intraperitoneal 5-FU chemotherapy efficacy further, an adjuvant approach is attainable. Immediate postoperative chemotherapy after pancreatectomy, could be possible to perform to attack small residual disease and microscopic peritoneal metastases.

10. Considerations for the future

The simple procedure of intraperitoneal administration makes it realistic to offer treatment in the patients home, with the aid of a nurse. The abdominal discomfort and nausea could be reduced by the use of oral analgesics and antiemetics. To further reduce the impact on the daily life of the patients, the intraperitoneal 5-FU treatment could be initiated in the evening, thus reducing the discomfort of a distended abdomen.

Administering 5-FU in late evening could also have an impact on toxicity. During continuous infusion, the highest 5-FU plasma concentrations were found in early morning (*Levi et al. 2004*). The rationale advocated for chronomodulated 5-FU therapy has been the circadian rhythm in host drug tolerance. The DNA synthesis in bone marrow and mucosa is lowest around midnight, implying the possibility to give a higher dose of 5-FU at night than at daytime, without increased toxicity. Chronomodulation of infusional 5-FU, with the maximal dose administered during the night when the proliferation of normal target tissue is at nadir, increased the haematological tolerability (*Penberthy et al. 2001*)).

The survival of patients with liver metastases given intraperitoneal 5-FU was 6.3 months. A more aggressive treatment of patients with liver metastases could include the use of intraperitoneal cisplatinumbased chemotherapeutic drugs. 5-FU acts synergistic with cisplatin, due to impaired repair of cisplatin-caused DNA-injury. The experience with intraperitoneal cisplatin is substantial in treatment of ovarian cancer, and side-effects are tolerable. In recent studies in metastatic pancreas cancer the addition of cisplatin or oxaliplatin IV to different chemotherapeutics had beneficial effect on tumour response (*El Rayes et al. 2003; Kulke et al. 2004; Novarino et al. 2004; Wang et al. 2002*). Cisplatin, like 5-FU, has a favourable peritoneal to plasma ratio, and also the ability to act systemically after absorption from the abdominal cavity (*Howell et al. 1990*). Intraperitoneal cisplatin and 5-FU would be an interesting combination treatment for non-resectable pancreas cancer, especially in patients who accept a higher toxicity.

To further sharpen the instrument, with which the most appropriate chemotherapeutic drug is chosen, it could be beneficial to exclude patients that suffer a risk of serious adverse effects of 5-FU or patients with tumours less sensitive to 5-FU. DPD is not routinely measured in patients even though 2–3% in the populations have some degree of deficiency (*Chazal et al. 1996*). In patients that lack DPD, the toxic effect is considerable and definitely calls for stopping the treatment. Analysing DPD activity in peripheral mononuclear blood cells has been proposed to identify patients at risk of developing severe toxic reactions. Around 80% of 5-FU is catabolised in the liver, and normal blood DPD activity dose not necessary reflect the patients total catabolic ability (*Stephan et al. 1995*). If patients that lack DPD are identified and excluded, the intraperitoneal dose of 5-FU could most likely be increased further in future protocols.

In a treatment where the PAC is placed by laparoscopy or laparotomy, there is a possibility to obtain tumour tissue samples. Analysing thymidylate synthase (TS) activity in the tumour, it is possible to direct 5-FU therapy to patients that presumably have the best response. TS is a key enzyme in the synthesis of pyrimidine in the *de novo* pathway of DNA synthesis and a major target of 5-FU. Antimetabolite agents directed against TS, as TAS-102, has effect *in vitro* on 5-FU non-sensitive cancer cell lines (*Emura et al. 2004*). Exhibiting high levels of TS in the tumour, correspond to longer survival in patients receiving adjuvant 5-FU after curative resection (*Takamura et al. 2002*).

It is important to explore new means of defining endpoints for treatment, so that treatment can be terminated before the side effects overweigh the symptoms from the progressive disease. The glycoprotein CA 19-9 (*Koprowski et al. 1981*) is elevated in patients with pancreas cancer. After preoperative radiotherapy and following laparotomy with the intent to perform curative resection, it was found that a marked increase of CA 19-9 after radiotherapy, correlated to development of early metastases (*Ohara et al. 2001*). CA 19-9 has been proposed to serve as an early indicator of response to chemotherapy (*Ziske et al. 2003; Miella et al. 2004*) or as a detector of recurrence after therapy (*Micke et al. 2003*). To monitor CA 19-9 changes during therapy, could help to decide when to stop treatment, or move on to second-line therapy.

FDG-PET (18-fluoro-deoxy-glucose positron emission tomography) can be used as an therapeutic marker, because the functional changes in pancreas cancer cells caused by enhanced glucose metabolism. The normal pancreas tissue is not visible on PET scan (*Berberat et al. 1999*). Even in detecting the presence of a tumour, the PET has shown to be more sensitive than CT (*Yoshioka et al. 2004*). It has also been shown that if the FDG uptake fade out after a month of treatment, the survival of the patient is longer than if the tumour still takes up FDG. Persistent glucose uptake during treatment seems to be a bad sign (*Maisey et al. 2000*), and this could call for an early change of therapy, instead of waiting for visible tumour progress on the CT scan.

To overcome the bias of patient selection, and explore the survival benefit in conjunction with the low toxicity profile of intraperitoneal 5-FU treatment, a randomised study would be proper to perform. Intraperitoneal 5-FU should be tested against intravenous gemcitabine monotherapy, as more toxic combinations has failed to yield significant survival benefit.

11. Conclusions

Experimental studies

It is possible to estimate peritoneal blood flow changes caused by IV vasopressin with the ^{133}Xe-clearance technique.
Peritoneal carcinomatosis does not significantly alter the ^{133}Xe-clearance.
Intraperitoneal 5-FU administration decreases the vasopressin induced ^{133}Xe-clearance reduction, 1–2 days after intraperitoneal administration.
Peritoneal carcinomatosis dose not affect the vasopressin induced ^{133}Xe-clearance reduction.

Clinical studies

In patients with non-resectable pancreas cancer, intraperitoneal 5-FU up to 1250 mg/m^2 for two days every third week can be given without WHO grade 3 and 4 toxicity.
The treatment is well tolerated with few and minor side effects.
With intraperitoneal 5-FU and intravenous Leucovorin treatment tumour responses can be achieved.
Addition of vasopressin does not enhance the pharmacokinetics of intraperitoneal 5-FU to the extent that it motivates the increase in side effects. Vasopressin significantly decreased 5-FU Cmax but not AUC in plasma. The 5-FU plasma concentrations measured after intraperitoneal 5-FU are in the same range as concentrations after continuous IV infusions over days.

12. Popularised Summary in Swedish

I Sverige upptäcks årligen knappt 900 fall av cancer i bukspottskörteln (pankreascancer). Eftersom cancern vid diagnos ofta är i långt framskridet skede, är det endast möjligt att operera bort tumören hos cirka 20% av patienterna. Fem år efter operationen lever endast 25% av de opererade patienterna. För patienter där operation inte är möjlig är framtidsutsikterna utomordentligt dystra. De flesta patienterna är döda i sin sjukdom efter 4–6 månader. Vissa patienter kan erbjudas stödjande och lindrande cellgiftsbehandling. Överlevnaden förlängs då till 6–9 månader.

Tumörer såsom pankreascancer, som brukar tillväxa lokalt runt bukspottskörteln eller i bukhålan och inte på spridda ställen i kroppen, kan vara lämpliga att behandla med cellgift som ges regionalt. Om cellgiftet ges in i bukhålan, istället för i blodet (intravenöst) utsätts tumören lokalt för höga koncentrationer av cellgiftet. Mycket av cellgiftet, som sedan tas upp i kroppen, passerar levern, där det bryts ned. Det innebär att halten cellgift i blodet blir lägre än om motsvarade mängd hade givits intravenöst, varvid biverkningarna minskar.

Genom djurförsök vet vi att om 5-Fluorouracil (5-FU), ett cellgift som angriper arvsmassan och dess kopiering vid celldelningen, ges i bukhålan istället för i blodet så uppnås 5 ggr högre koncentration i de lymfbanor som leder från bukhålan och över 250 ggr högre koncentration i bukvätskan än i blodet. Cancerceller delar sig oftare än normala celler och är därför känsligare för 5-FU. Vasopressin är ett hormon som ger en kraftig kärlsammandragning i de blodkärl som omger bukhålan. Drogen har haft en viktig användning vid behandling av blödande åderbråck i matstrupen. Om man ger vasopressin, samtidigt med cellgiftet, kan man öka cellgiftets koncentration i bukhålan och i lymfbanorna och på så sätt nå de lokala spridningsvägarna, utan att öka biverkningarna. Ett tredje läkemedel som vi använt oss av, Leucovorin, ökar effekten av 5-FU mot cancern.

Laboratorieförsök

Xenon är en ädelgas som inte reagerar med kroppens celler. En av dess radioaktiva isotoper Xenon-133 (^{133}Xe) är enkel att hantera och lämpar sig för laboratoriebruk. Strålningen från isotopen är svag, enkel att skydda sig från, och är lätt att mäta. ^{133}Xe som sprutas in i bukhålan, transporteras därifrån genom blodflödet och förs ut ur kroppen med utandningsluften. Om blodflödet minskar, som vid kärlsammandragning, sköljs ^{133}Xe långsammare bort från mätområdet. Påverkas blodflödet av andra faktorer, som av cellgift i bukhålan eller tumörer på bukhinna, syns detta också på hur snabbt ^{133}Xe sköljs bort. På så sätt kan man uppfatta ^{133}Xe borttransport som ett mått på blodflödet. Mätningarna gjordes med ^{133}Xe metoden, men som jämförelse använde vi också en Laser doppler mätare applicerad direkt på bukhinnan för att mäta blodflödesförändringar.

Frågeställningarna var om ^{133}Xe metoden kan uppskatta blodflödet i bukhålan och om vasopressin kan minska detta blodflöde. Vidare ville vi ta reda på om 5-FU

givet i bukhålan eller om tumörer på bukhinnan påverkar blodflödet eller blodflödets reaktion på vasopressin.

Kliniska studier

Patienter med cancer i bukspottskörteln, som inte kan opereras bort, behandlades med 750, 1000, 1250 eller 1500 mg/m^2 5-FU i 2 liter koksaltlösning givet in i bukhålan. Behandlingen gavs under två dagar var tredje vecka. De patienter som behandlades med 750 mg/m^2 5-FU gavs samtidigt vasopressin 0.1 enheter/minut under 3 timmar, antingen på den första eller andra behandlingsdagen. Blodprov för analys av 5-FU koncentrationen togs var 30 minut under fem timmar. Välmående (Karnofsky Index), kroppsvikt och förbrukning av smärtstillande medel av morfintyp registrerades vid varje behandling.

Efter fyra behandlingar, var tredje månad, utfördes en datortomografi av buken. Om tumören inte hade vuxit fortsatte behandlingen ytterligare tre månader. Behandlingen avslutades om tumören ökade i storlek, om patienten fick svår blodcellspåverkan eller blev påtagligt försämrad i sitt välbefinnande. Patienten erbjöds då bedömning av onkologläkare för behandling med cellgifter givet i blodet (intravenöst). Om inte det blev aktuellt, gavs bästa möjliga symptomlindrande omvårdnad.

Frågeställningarna i de kliniska studierna var, om behandling med 5-FU givet i bukhålan till patienter där det inte var möjligt att operera bort cancer i bukspottkörteln, var genomförbar med hänsyn till biverkningar och livskvalitet, och om behandlingen påverkade tumören. Vidare ville vi veta om vasopressin, påverkade cellgiftets fördelning i kroppen (farmakokinetik) så att effekten ökade och biverkningarna minskade.

Resultat laboratorieförsök

Med ^{133}Xe-metoden kunde blodflödet i bukhålan och blodflödesförändringar orsakade av vasopressin registreras. Laser doppler metoden registrerade en motsvarande blodflödesminskning i bukhinnan. Om man gav 5-FU i bukhålan påverkades inte det basala blodflödet men vasopressin kunde inte sänka blodflödet under två dagar efter behandlingen. Tumörväxt i bukhålan påverkade inte blodflödet eller vasopressin effekten.

Resultat kliniska försök

Det var möjligt att med mycket lite biverkningar och med få komplikationer ge 5-FU i bukhålan under lång tid. Även om ingen direkt jämförelse kunde göras så förefaller överlevnadstiderna lika bra som vid intravenös cellgiftsbehandling. Vasopressin gav inga uppenbara positiva effekter på 5-FU fördelning i kroppen, och vasopressin föreföll inte heller öka effekten av cellgiftet. Däremot ökade biverkningarna när vasopressin användes.

Sammanfattningsvis förfaller 5-FU givit i bukhålan, kunna påverka blodflödet i bukhinna så att den kärlsammandragande effekten av vasopressin minskar. Det är därför troligt att vasopressin inte förbättrar fördelningen av 5-FU i kroppen. Tumörer på bukhinnan påverkar däremot inte blodflödet. Det är enkelt att ge 5-FU i bukhålan på patienter med cancer i bukspottkörteln. Biverkningarna är få och lindriga. En tumörhämmade effekt noteras.

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