Feasibility of Veno-Venous Bypass Surgery Using Leukocyte Adhesion Filters during Abdominal Surgery in a Porcine Model

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Key Words

Leukocyte adhesion filters, porcine model · Leukocyte depletion filter · Leukocyte depletion filter integration, safety study · Oncologic surgery

Abstract

During oncologic surgery, manipulation of tumour tissue is almost unpreventable; liver resection even carries a higher risk of tumour cell dissemination into venous blood. Under in vitro conditions, a tumour cell reducing effect of some leukocyte adhesion filter systems has been shown. In a preclinical porcine model, these filters were used as integrated parts of a veno-venous bypass system used for liver surgery, run by a biopump. Practicability, handling and safety aspects of the filter system were analysed; the system was easy and safe to handle, and treated animals survived without any complications. For the future, effectiveness of the tumour cell depletion has to be examined in further experimental and clinical studies.

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Introduction

Manipulation of tumour tissue during surgical resection of gastrointestinal malignancies often leads to tumour cell dissemination into the systemic blood circulation [1]. This may influence the prognosis of malignant diseases by local tumour recurrence and formation of distant metastasis [2, 3]. Concepts to prevent intraoperative tumour cell dissemination were described already in the 1960s by the so-called ‘no-touch surgical technique’, gained by early lymph-vascular closure before preparation and resection of the primary colorectal cancer [4].

In patients undergoing liver resection due to malignancies, prevention of intraoperative tumour cell dissemination is difficult to obtain. Compared to surgical procedures for primary colorectal carcinoma, liver resection results in a higher incidence of mechanically induced intraoperative tumour cell dissemination [1]. This might be caused by the anatomy and the need of extensive mobilisation before possible occlusion of the venous drainage. As the surgical mobilisation is unpreventable in commonly used surgical techniques, methods of tumour cells depletion from the systemic circulation should be discussed. Therefore, cell filtration of venous blood drained from tumour tissue could be a useful option. Previous studies reported that some filters used for leukocyte

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Fig. 1. Experimental setting, catheter placement and tube connection (1 = cannula in common iliac vein; 2 = biopump; 3 = filter; 4 = cannula in jugular vein; 5 = clamp on IVC).

depletion are able to remove tumour cells under in vitro conditions [5]. Comparable filter systems are used in cardiac surgery combined with a heart-lung machine and in blood banks for preparation of stored blood units.

We present a preclinical safety study concerning the integration of a leukocyte depletion filter (Leukogard LG-6, Pall®) into an extracorporeal veno-venous bypass (biopump, BioMedicus®) between the inferior caval vein (ICV) and subclavian vein, using a porcine animal model. Clinical side effects, critical leukocyte depletion, and technical handling of the filter system were analyzed.

Materials and Methods

Animals and Study Groups

The study was performed in 10 female German Landrace pigs with a mean body weight (BW) of 35 ± 6 kg, under protocol approved by the local committee of animal use and care. Pigs were sacrificed in the post-operative course at 2 h (group 1, $n = 4$) and 7 days (group 2, $n = 6$) with post-mortem examination.

Anaesthesia

Premedication in all animals was done by intramuscular application of 10 mg/kg BW azaperone and 0.025 mg/kg BW atropine sulphate. For oral intubations the animals received thiopental (12.5 mg/kg BW) and fentanyl (0.005 mg/kg BW) intravenously. After intubations animals were ventilated by intermittent positive pressure with 1/3 oxygen using a Ventillog respirator (Dräger, Lübeck, Germany). The tidal volume was kept between 300 and 450 ml with a ventilation rate between 10 and 15 per minute. Thiopental was administered continuously and boli of fentanyl were given to maintain the narcosis.

Surgical Procedure

The left subclavian vein was canalised with the efferent 'flow return catheter' (14 F, Medos®) after surgical dissection. The abdominal cavity was opened through a midline laparotomy. The left common iliac vein was mobilized, controlled by a tourniquet and afterwards canalised within the IVC with the afferent catheter of the system (14 F, Medos®). The heparin-coated bypass system (BioMedicus®) was prepared and flushed with saline solution. Two leukocyte adhesion filters (Leukogard LG-6, Pall®) were integrated into the bypass circuit (fig. 1). Prior to extracorporeal bypass circulation, the subdiaphragmatic suprahepatic IVC was closed using a Satinsky clamp; major surgical mobilisation of the liver was avoided. After tube connection of the extracorporeal system, the flow rate was kept between 0.5 and 1.5 l/min, equalising half of the cardiac output of the animals. The circulation time on the bypass was determined to 60 min; 30 min of filtration per filter. During filtration the portal vein was clamped within the hilum and the retrohepatic caval vein was isolated from the posterior abdominal wall, simulating a liver mobilisation procedure.

Finishing bypass circulation, the Satinsky clamp was released and bypass catheters were removed to obtain the physiological blood flow. After final haemostasis the abdomen was closed with a running 2/0 PDS suture. Animals of group 2 were extubated at the end of general anaesthesia; further observation was done at the animal care unit.

Parameters

Blood samples for determination of WBC gradient across the filter (inflow vs. outflow) and systemic WBC, platelet count, blood coagulation parameters (PTT, TPZ, ATIII), haemolysis parameters (free haemoglobin, haptoglobin count) were taken prior to operation and during the bypass circulation after 10, 20, 30, 40, 50 and 60 min; in animals of group 2 additionally on each post-operative day.

Blood gas analysis, glucose and electrolyte status were monitored hourly. Haemodynamic and respiratory parameters (arterial blood pressure, central venous pressure, heart rate, respirator ventilation) were monitored continuously.

Table 1. Mean value and standard deviation of heart rate (HR), mean arterial blood pressure (MAP), central venous blood pressure (CVP), oxygenation (pO₂) during the study (pre-operative, 10, 20, 30, 40, 50, 60 min after starting the veno-venous bypass, post-operative)

	HR/min	MAP/mm Hg	CVP/mm Hg	pO ₂ /mm Hg
Pre-operative	97 ± 20	45 ± 21	3 ± 1.0	169 ± 61
10 min	114 ± 29	44 ± 11	3 ± 0.6	170 ± 57
20 min	110 ± 18	54 ± 8	6 ± 1.0	174 ± 61
30 min	102 ± 34	53 ± 6	5 ± 1.0	176 ± 43
40 min	101 ± 35	54 ± 3	9 ± 2.0	172 ± 30
50 min	103 ± 31	60 ± 14	14 ± 3.6	174 ± 27
60 min	100 ± 30	56 ± 4	11 ± 3.6	173 ± 24
Post-operative	119 ± 11	58 ± 8	14 ± 3.5	161 ± 43

Statistical Analysis

Statistics of white blood cell count and cardiopulmonary parameters were performed by SPSS 10.0 using the paired Student's test. A p value of $p < 0.05$ was considered to be statistically significant.

Results

Surgical Procedure

All animals survived the surgical procedure and extracorporeal blood filtration. The implementation of the bypass system with integrated leukocyte depletion filters was technically easy and uneventful. The clamping of the infrahepatic IVC was possible without major mobilisation of the liver by using a large Satinsky clamp. The cardiopulmonary situation remained stable during extracorporeal bypass circulation (0.5 up to 1.5 liters/min) in all animals, catecholamine treatment was not necessary. Minor changes of heart rate, arterial and central venous blood pressure and oxygenation during the experimental time course are shown in table 1. The mean operation time was 130 min, including the filtration period. The surgical blood loss was <200 ml in each case; the blood loss due to dead-space volume of the tubing system was 150 ml.

Safety and Efficacy of the Filter System

During extracorporeal filtration no haemolytic reactions were noticed, counts of free haemoglobin and haptoglobin remained within a normal range. Clotting parameters were also unaffected. The removal of flow catheters was uneventful, vascular thrombosis or tube clotting was not observed.

An expected significant intraoperative reduction of the WBC after starting filter passage was observed in all animals ($n = 10$). After 30 min of filtration, the mean WBC count of approximately 6,500 WBC/ μ l blood dropped to mean values of 4,000 WBC/ μ l blood. The WBC gradient

Fig. 2. Systemic WBC (mean values and standard deviation) during the operation in 10 animals; filter replacement after 30 min.

across the filter decreased significantly during the filtration period. After a second period of filtration (30 min) using the next filter, the WBC count dropped to mean value of 3,000 WBC/ μ l blood (fig. 2), again paralleled by a time depending on decreasing WBC gradient across the filter.

Post-Operative Follow-Up

All animals of group 2 ($n = 6$) survived the 7-day follow-up without any complications. Surgery- or leucopenia-related infections did not occur over the whole observation period. The WBC increased to physiologic values in all animals within 4 days following operation (fig. 3).

Post-mortem examinations showed neither ischaemic nor congestion characteristics of intra-abdominal organs. Thrombosis and infectious foci were not observed.

Fig. 3. Systemic WBC (mean values and standard deviation) after operation (day 0) and during follow-up period (days 1–7) in 6 animals.

Discussion

The usage of leukocyte depletion filters is established in different clinical disciplines. They are often used in extracorporeal circulation systems of heart-lung machines in order to reduce the ischaemic reperfusion injury.

The presented study shows the feasibility of using a leukocyte depletion filter in an extracorporeal circuit for abdominal surgical procedures. To this purpose we established a safety study in a large animal model using a veno-venous bypass (ICV to subclavian vein). All animals tolerated clamping of IVC and consecutive filtration on the bypass with a biopump for 60 min without complications. Cardiopulmonary function was documented to be stable for heart rate, arterial blood pressure, central venous pressure and oxygenation.

Blood samples demonstrate a significant reduction of WBC count during filtration as expected, critical leucopenia was not observed. The maximum WBC decrease was detected within the first minutes of filtration; further WBC decrease was possibly limited by a filter medium saturation kinetic. According to the manufacturer's data, exhausting filter capacity was seen within the first 30 min of filtration. Baksaas et al. [6] demonstrated comparable effects in an in vitro model of extracorporeal circulation.

The early post-operative recovery of WBC in animals of group 2 was seen as a rebound phenomenon, possibly due to post-operative stress. No animal had to be treated in the post-operative course due to infectious complications.

Though capacity of leukocyte removal has been improved by evolving filtration technologies, most of the

mechanisms of cell removal remain unclear [7–10]. Hydrophilicity [11], surface tension, charge density of fibres [12] and filtration temperature [13, 14] are discussed as co-factors of cell removal. The depletion of selected white blood cells is based on trapping in the fibre network [15], activation and subsequent adhesion to the fibres [16, 17].

For safety reasons, the coagulation cascade should not be activated during filter medium passage. As previously shown by Gu et al. [18], an activation of complement factors or uprising of elastase or thromboxane concentrations cannot be observed by the use of polyester-based filter systems. Changes in the coagulation parameters were also not observed in our investigation by the use of the polyester fibre-based filter (Leukogard LG-6, Pall®).

It still remains unclear which mechanisms are involved in consecutive tumour cell depletion; comparable mechanisms to leukocyte removal have to be discussed. However, due to their different entities, individual and heterogeneous population they are difficult to investigate. Edelman et al. [19] could prove a tumour cell depleting effect using isolated tumour cells of urothelium carcinoma for the leukocyte filter system RC-400® under in vitro conditions. Perseghin et al. [20] reported a similar observation in patients with lung carcinoma after filtering cell saver blood from the surgical site.

We previously reported that the medium of the leukocyte depletion filter used in this study reduces the number of native human tumour cells of colorectal carcinoma from blood group-compatible full blood under in vitro conditions of in-line filtration [5]. This animal model shows that the same filter medium is safe for a possible use as an integrated part of veno-venous bypass systems in extended oncologic surgery.

Due to these results, clinical studies in selected patients can be justified if a veno-venous bypass system is needed for extended liver resections.

References

- 1 Weitz J, Koch M, Kienle P, Schrodel A, Willeke F, Benner A, et al: Detection of hematogenic tumor cell dissemination in patients undergoing resection of liver metastases of colorectal cancer. *Ann Surg* 2000;232:66–72.
- 2 Weitz J, Kienle P, Lacroix J, Willeke F, Benner A, Lehnert T, et al: Dissemination of tumor cells in patients undergoing surgery for colorectal cancer. *Clin Cancer Res* 1998;4:343–348.
- 3 Lindemann F, Schlimok G, Dirschedl P, Witte J, Riethmüller G: Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. *Lancet* 1992;340:685–689.
- 4 Turnbull RB Jr, Kyle K, Watson FR, Spratt J: Cancer of the colon: The influence of the no-touch isolation technic on survival rates. *Ann Surg* 1967;166:420–427.
- 5 Frühauf NR, Dumpich O, Kaudel CP, Kasimir-Bauer S, Oldhafer KJ: Filtration of malignant cells: Tumour cell depletion in an ex vivo model using a leukocyte adhesion filter. *Perfusion* 2001;16(suppl):51–55.
- 6 Baksaas ST, Videm V, Mollnes TE, Pedersen T, Karlsen H, Svennevig JL: Effects on complement, granulocytes and platelets of a leukocyte-depletion filter during in vitro extracorporeal circulation. *Scand Cardiovasc J* 1997;31:73–77.
- 7 Dzik S: Leukodepletion blood filters: Filter design and mechanisms of leukocyte removal. *Transfus Med Rev* 1993;7:65–77.
- 8 Sniecinski I, O'Donnell MR, Nowicki B, Hill LR: Prevention of refractoriness and HLA alloimmunization using filtered blood products. *Blood* 1988;71:1402–1407.
- 9 Pietersz RN, van der Meer PF, Seghatchian MJ: Update on leucocyte depletion of blood components by filtration. *Transfus Sci* 1998;19:321–328.
- 10 Legrand E, Vincent M: Characteristics and mechanisms of action of leukocyte depletion filters (in French). *Rev Fr Transfus Hemobiol* 1993;36:211–221.
- 11 Bruil A, Sheppard JI, Feijen J, Feuerstein IA: In vitro leukocyte adhesion to modified polyurethane surfaces. III. Effect of flow, fluid medium, and platelets on PMN adhesion. *J Biomater Sci Polym Ed* 1994;5:263–277.
- 12 Krailadsiri P, Seghatchian J: Negatively charged leukocyte filter significantly enhances kallikrein and thrombin-like activities of platelet concentrates. *Thromb Res* 1996;83:469–474.
- 13 Beaujean F, Segier JM, le Forestier C, Duedari N: Leukocyte depletion of red cell concentrates by filtration: Influence of blood product temperature. *Vox Sang* 1992;62:242–243.
- 14 Eastlund T, Charbonneau T, Britten A: Changes in neutrophil aggregation and chemotaxis response during 18-hour cold storage. *Transfusion* 1984;24:513–515.
- 15 Steneker I, van Luyn MJ, van Wachem PB, Biewenga J: Electron microscopic examination of white cell reduction by four white cell-reduction filters. *Transfusion* 1992;32:450–457.
- 16 Shiba M, Tadokoro K, Sawanobori M, Nakajima K, Suzuki K, Juji T: Activation of the contact system by filtration of platelet concentrates with a negatively charged white cell-removal filter and measurement of venous blood bradykinin level in patients who received filtered platelets. *Transfusion* 1997;37:457–462.
- 17 Buescher ES, Gallin JI: Effects of storage and radiation on human neutrophil function in vitro. *Inflammation* 1987;11:401–416.
- 18 Gu YJ, Obster R, Haan J, Gallandat Huet RC, Eijgelaar A, van Oeveren W: Biocompatibility of leukocyte removal filters during leukocyte filtration of cardiopulmonary bypass perfusate. *Artif Organs* 1993;17:660–665.
- 19 Edelman MJ, Potter P, Mahaffey KG, Frink R, Leidich RB: The potential for reintroduction of tumor cells during intraoperative blood salvage: Reduction of risk with use of the RC-400 leukocyte depletion filter. *Urology* 1996;47:179–181.
- 20 Perseghin P, Vigano M, Rocco G, Della Pona C, Buscemi A, Rizzi A: Effectiveness of leukocyte filters in reducing tumor cell contamination after intraoperative blood salvage in lung cancer patients. *Vox Sang* 1997;72:221–224.