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.

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...
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 $35 \pm 6$ 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 Ventilog 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 subdiaphragmal 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)

<table>
<thead>
<tr>
<th>Time</th>
<th>HR/min</th>
<th>MAP/mm Hg</th>
<th>CVP/mm Hg</th>
<th>pO2/mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-op</td>
<td>97 ± 20</td>
<td>45 ± 21</td>
<td>3 ± 1.0</td>
<td>169 ± 61</td>
</tr>
<tr>
<td>10 min</td>
<td>114 ± 29</td>
<td>44 ± 11</td>
<td>3 ± 0.6</td>
<td>170 ± 57</td>
</tr>
<tr>
<td>20 min</td>
<td>110 ± 18</td>
<td>54 ± 8</td>
<td>6 ± 1.0</td>
<td>174 ± 61</td>
</tr>
<tr>
<td>30 min</td>
<td>102 ± 34</td>
<td>53 ± 6</td>
<td>5 ± 1.0</td>
<td>176 ± 43</td>
</tr>
<tr>
<td>40 min</td>
<td>101 ± 35</td>
<td>54 ± 3</td>
<td>9 ± 2.0</td>
<td>172 ± 30</td>
</tr>
<tr>
<td>50 min</td>
<td>103 ± 31</td>
<td>60 ± 14</td>
<td>14 ± 3.6</td>
<td>174 ± 27</td>
</tr>
<tr>
<td>60 min</td>
<td>100 ± 30</td>
<td>56 ± 4</td>
<td>11 ± 3.6</td>
<td>173 ± 24</td>
</tr>
<tr>
<td>Post-op</td>
<td>119 ± 11</td>
<td>58 ± 8</td>
<td>14 ± 3.5</td>
<td>161 ± 43</td>
</tr>
</tbody>
</table>

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/µl blood dropped to mean values of 4,000 WBC/µl blood. The WBC gradient 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/µl 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. Baksas 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.
Feasibility of Leukocyte Adhesion Filters in Pigs

References