Anterograde versus retrograde isolated lung perfusion with melphalan in the WAG-Rij rat

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Abstract

Objective: Isolated lung perfusion (ILuP) is an experimental technique currently tested to increase the 5-year survival of 40% after surgical resection of pulmonary metastases from certain solid tumors. The standard technique of anterograde perfusion was compared with retrograde isolated lung perfusion in which the drug is introduced through the pulmonary veins while the effluent is collected from the pulmonary artery. Since the lung has a dual arterial circulation through the pulmonary artery and bronchial circulation, perfusion through the pulmonary veins can result in a more homogeneous distribution throughout the lung with subsequent higher melphalan concentration.

Methods: We randomized 20 rats into two groups. Group one underwent anterograde isolated left lung perfusion while group two underwent retrograde isolated left lung perfusion. A dose of 2 mg/kg melphalan (MN) was administered to the lung at a flow of 0.5 mL/min during 30 min, followed by a 5-min washout with buffered hetastarch (BHE). The final melphalan lung concentration (FMLC) was determined in the hilum, at the apex, the mid-periphery and the base of the lung. Statistical analysis was done with an unpaired student’s t-test.

Results: Retrograde left ILuP resulted in a higher FMLC in the hilum (\(P<0.0001\)) and in the base of the lung (\(P<0.03\)), while anterograde ILuP induced a higher concentration at the apex of the lung (\(P<0.04\)). No difference was seen in the mid-peripheral area of the lung (\(P>0.92\)).

Conclusions: In this experimental study, retrograde perfusion seems to increase final melphalan lung concentration in hilar and basal regions of the lung compared to anterograde perfusion.

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Keywords: Isolated lung perfusion; WAG-Rij rat; Melphalan; Pulmonary artery perfusion; Pulmonary metastases

1. Introduction

Isolated lung perfusion (ILuP) is an experimental technique currently tested to increase the 5-year survival of 40% after surgical resection of pulmonary metastases from certain solid tumors [1].

The standard technique of isolated lung perfusion is by anterograde perfusion. The drug is administered through the pulmonary artery while the effluent is collected at the venous puncture site. Although animal studies demonstrated superior pharmacokinetics and prolonged survival, compared to intravenous therapy, optimal perfusion parameters are unknown.

Since the lung has a dual blood supply by the pulmonary arterial and bronchial circulation, anterograde perfusion can result in a decreased perfusion of certain areas of the lung mainly perfused by the bronchial circulation. Retrograde isolated lung perfusion in which the drug is introduced through the pulmonary veins while the effluent is collected from the pulmonary artery can result in a more homogeneous distribution throughout the lung with subsequent higher melphalan concentration.

The aim of the current experiment is to evaluate which perfusion method will result in higher melphalan lung levels, anterograde or retrograde perfusion, and to compare the highest melphalan lung levels in both groups in different areas of the lung.

2. Materials and methods

2.1. Animals

Male inbred WAG-Rij strain rats (weight, approximately 200 g), obtained from Harlan-CPB (Zeist, The Netherlands), were used for all experiments. Animals were treated in accordance with the Animal Welfare Act and the 'Guide for the Care and Use of Laboratory Animals' (NIH Publication 86-23, revised 1985). The rats were transported in sterile conditions, housed in suspended mesh wired cages and fed a standard pellet diet ad libitum (standard rat chow, Hope Farms, Woerden, The Netherlands). The Institutional Animal
Care Use Committee, University Hospital Antwerp, approved the experimental protocols.

2.2. Single-pass anterograde and retrograde isolated left lung perfusion

In both groups anesthesia was induced with isoflurane 4% (Forene®, Abbott) in a mixture of N₂O and O₂ (3:1). After 5 min of incubation, intubation was performed by translaryngeal illumination according to the technique described by Hendriks et al. [2]. Once connected to the ventilator, the N₂O:O₂-ratio was set to 1:1 and isoflurane was titrated between 1 and 1.5%. Ventilation was accomplished with a volume-controlled ventilator at a rate of 65/min and a tidal volume of 10 mL/kg. After a left thoracotomy, the left lung is positioned anteriorly and the hilum is dissected free from the backside. Both pulmonary vein and artery are clamped with microvascular clips. Perfusion was done with 2-mg/kg melphalan (MN) in buffered hetastarch (BHE), at a flow of 0.5 mL/min during 30 min, followed by a 5-min washout with BHE.

For the anterograde perfusion group, a PE-10 perfusion catheter is inserted into the pulmonary artery. The chemotherapy will circulate by means of a roller pump and the effluent is collected at the venous site.

For the retrograde perfusion group, the PE-10 perfusion catheter is inserted at the junction of the pulmonary veins with the left atrium. The chemotherapy will circulate by means of a roller pump and the effluent is collected at the pulmonary artery. The bronchial circulation is interrupted by ligation in order to prevent systemic leakage. After ILuP, the rat was killed by a venous cut down of the superior caval vein.

The perfusate was controlled at 37 °C throughout the duration of perfusion. Rats were placed on a heating pad immediately after induction and body temperature was kept constantly between 34 and 37 °C.

2.3. MN processing and measurement

Gas chromatography–mass spectrometry, as described by De Boeck et al. [3], was used for measuring melphalan levels in lung tissue and serum. P-[Bis(2-chloroethyl) amino]phenylacetic acid methyl ester was used as an internal standard. Samples were extracted over trifunctional C18 silica columns.

2.4. Experiment

Twenty rats were randomized into two groups. For both the anterograde and the retrograde group the left lung was excised after the perfusion and a 5-min washout. The lung was divided into four regions, i.e. a hilar region and an apical, middle and a basal region in the periphery. The periphery depicts the outer two-third of the lung while the hilar region is the inner third.

2.5. Statistical analysis

All data are presented as mean ± SD. Statistica 5.5 (StatSoft, Inc., Tulsa, USA) was used for the statistical analysis. Shapiro-Wilk’s was used to evaluate normality and an unpaired student’s t-test was used to compare anterograde with retrograde perfusion in the four regions of the left lung. Significance was defined as P < 0.05.

3. Results

Procedure related mortality was 0%.

3.1. Final melphalan lung concentration

According to Shapiro-Wilk’s test (SW-W: 0.93, P = 0.60) the data have a normal distribution. Therefore, an unpaired student’s t-test is used for further analysis. Table 1 depicts the final melphalan lung concentration for both the anterograde and retrograde perfusion in the four different regions.

Retrograde perfusion resulted in a higher FMLC in the hilar region (P < 0.0001) and at the base of the periphery (P = 0.03). Anterograde perfusion resulted in higher concentration at the apical peripheral region (P = 0.04). In the mid-peripheral region there is no difference between the directions of perfusion (P = 0.92).

4. Discussion

Isolated lung perfusion (ILuP) is an experimental technique currently tested to increase the 5-year survival of 40% after surgical resection of pulmonary metastases from certain solid tumors [1]. Theoretically, it has the advantage of delivering chemotherapy in a high dose to the lung while avoiding systemic exposure and metabolism through the liver or kidneys. ILuP has proven to be superior compared to intravenous administration for the treatment of pulmonary metastases in small and large animal models [4–8]. Clinical trials were started demonstrating isolated lung perfusion to be technically feasible [9,10], also in combination with a complete metastasectomy [11].

The standard technique of isolated lung perfusion used in animal experiments and clinical trials is by anterograde perfusion. With this technique, the drug is admitted through the pulmonary artery while the effluent is collected at the venous site into a closed circuit. Although most animal studies with the standard anterograde ILuP demonstrated superior pharmacokinetics and prolonged survival compared to intravenous therapy, the optimal route of perfusion and

<table>
<thead>
<tr>
<th>Region</th>
<th>FMLC° anterograde (µg/g)</th>
<th>FMLC° retrograde (µg/g)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hilar</td>
<td>18.84 ± 9.83</td>
<td>46.91 ± 13.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peripheral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apex</td>
<td>36.95 ± 8.43</td>
<td>27.85 ± 9.62</td>
<td>0.04</td>
</tr>
<tr>
<td>Mid</td>
<td>28.08 ± 17.49</td>
<td>28.84 ± 11.28</td>
<td>0.92</td>
</tr>
<tr>
<td>Basal</td>
<td>24.73 ± 13.00</td>
<td>38.05 ± 6.55</td>
<td>0.03</td>
</tr>
</tbody>
</table>

FMLC°, final melphalan lung concentration.

* Data are presented as mean ± SD.
ideal perfusion parameters are unknown. As shown by Milne et al. most pulmonary metastases are supplied by the pulmonary artery since their primary location is at the periphery of the lung. A minor part of these lung metastases will have a combined bronchial-pulmonary blood supply or unique bronchial blood supply. The bronchial artery dominantly supplies metastases located in the hilar region [12, 13]. Therefore, areas of the lung mainly perfused by the bronchial circulation are reached less by the standard technique of anterograde perfusion. In contrast with retrograde isolated lung perfusion by which the drug is introduced through the pulmonary veins, a more homogeneous distribution throughout the lung and subsequently higher melphalan concentration can be expected on a theoretical basis.

The technique of retrograde ILuP is not new but already described for flushing lung or lung-heart blocks for lung transplantation. For the treatment of pulmonary metastases, it is not widely accepted and it was only tested in an experimental setting for chemotherapeutics like doxorubicin and paclitaxel.

Schrump et al. displayed superior concentrations of paclitaxel in the lung after hyperthermic retrograde perfusion in a sheep model compared with intravenous administration [14]. Despite this finding retrograde perfusion was not compared with anterograde perfusion since this experiment was used to evaluate regional techniques for the treatment of primary neoplasms of the lung, which are mainly supplied by the bronchial circulation [12].

Krueger et al. compared anterograde with retrograde ILuP for the treatment of pulmonary metastases in the Fischer rat with doxorubicin. They could not demonstrate a significant difference between the doxorubicin levels of both groups. However, results did show a trend of higher doxorubicin concentration after retrograde perfusion [15].

Our experiment demonstrated that final melphalan lung levels were higher in some areas of the lung after retrograde perfusion, while in other areas higher melphalan lung levels were reached after anterograde perfusion. It is clear that isolated lung perfusion is still very complex and it is very difficult to achieve a homogeneous distribution in an organ that is relatively easily isolated on a theoretical basis. Further studies are needed to evaluate the possible benefit of retrograde perfusion. In our study it is demonstrated that some regions will be less reached by anterograde perfusion. This is most true for lesions at the hilar and basal parts of the lung.

References