Acute toxicity of irinotecan in the ex-vivo isolated perfused human lung model – high-dose therapy during isolated perfusion without acute toxic lung edema

Christian Biancosino\textsuperscript{a,b,*}, Marcus Albert*, Albert Linder\textsuperscript{a}

\textsuperscript{a}Department of Thoracic Surgery, Lungenklinik Hemer, Centre for Pneumology and Thoracic Surgery, Theo-Funckius-Str. 1, D-58675 Hemer, Germany
\textsuperscript{b}Department of General Thoracic Surgery, University Hospital of Freiburg, Freiburg, Germany

Received 19 January 2007; received in revised form 24 May 2007; accepted 26 May 2007

Abstract

In many cases unresectable or recurrent pulmonary metastases do not respond to systemic chemotherapy or the side-effects are not acceptable. Based on the results of our experiments the isolated lung perfusion could improve the option of local chemotherapy. Parameters for lung edema formation (relative increase in weight, gas exchange, histopathology) were evaluated during extracorporal ventilation and reperfusion of lobes resected for lung cancer. Drug concentration was measured in lung tissue, tumour and hilar lymphnodes. Irinotecan was detected in concentrations from 0.06 to 35.3 mg/g in correlation to the content of drug in the perfusate. None of the preparations perfused with a concentration up to 20 times higher than the concentration for systemic application generated a drug-dependent reperfusion edema. A toxic injury of lung parenchyma could be excluded histopathologically. Therefore, we documented that even a perfusion with 2000 mg/l does not cause any relevant acute toxic damages of the lung parenchyma. The transferability of pharmacological data gained through the IHLP is excellent and minimises potential adverse reactions for the patients during phase I trials. As an alternative to systemically applied cytostatic drugs the isolated lung perfusion with irinotecan deserves further attention due to its interesting pharmacological profile with regard to tumor selectivity.

Keywords: Isolated lung perfusion; Lung metastases; Anticancer drugs; Irinotecan

1. Introduction

Today resection and chemotherapy are being considered as a therapy for pulmonary metastases of different primary tumours. For many primary carcinomas a complete metastasectomy shows better long-term results, responsibility and side effects than chemotherapy alone. The 5-year survival rate ranges between 20% and 40%. That is much higher than expected after chemotherapy or radiotherapy alone [1].

It could be shown that systemic chemotherapy entailed tumor shrinkage in 35–50% of the patients, although it has had no major influence on the patients’ survival. Presumably the adverse reactions of the cytotoxic drugs had not allowed the therapeutic level to be reached [2]. For unresectable or recurrent pulmonary metastases – the supposed reasons for the latter ones are occult micro-metastases [3] – local high-dose chemotherapy by means of isolated lung perfusion is a promising therapeutic option, which is comparable to the isolated liver perfusion [4].

When treating pulmonary metastases and associated micrometastases a combination of resection and operative local perfusion appears to be the most advantageous procedure. Several groups reported about their clinical experience concerning the isolated lung perfusion [5, 6].

In the course of new studies, irinotecan (CPT-11) advanced to an important cytostatic drug in the treatment of colorectal carcinomas (particularly 5-fluorouracil-(5-FU) refractory ones [7, 8]), small cell and non-small cell lung cancers (NSCLC, SCLC), ovarian carcinomas and their metastases.

The interest in irinotecan is legitimate because it is metabolised to a stronger metabolite in hepatocytes as well as in tumour cells. Thereby it has also a higher efficiency because the drug is metabolised in the tumour itself. The necessary enzymes for inactivation are not present in tumour cells [9].

The ex-vivo isolated perfused human lung model which we have developed and used [10] is an important approach during the preclinical stage. It contributes to the establishment of therapeutical strategies and to the evaluation of in vivo pharmacokinetic conditions. Our model represents the in-vivo conditions in a better manner than could ever be realised in in-vitro experiments with human tumour cell culture or animal experiments.
2. Material and method

2.1. IHLP

We perfused anatomically resected lobes or pneumonectomy preparations due to bronchial carcinomas. Patients who had undergone a previous chemo- or radiotherapy were excluded. After cutting off the ligation of the veins the segmental, lobar and basal segmental arteries were cannulated within an ischemia time of < 45 min.

The preparations were perfused using a roller pump with a flow rate from 0.1 to 0.2 l/min. The mean pulmonary arterial pressure was kept constant below 25 mmHg. Intermit-tently we determined PAP, arterial and venous pH, PO2 and PCO2 in the perfusate [10].

The total volume of the perfusate was 1 l. The perfusate used was a physiological solution according to Perfadex®. The use of resected human lungs for perfusion was approved by the Ethics Committee of the University of Münster.

2.2. Perfusion quality

As the perfusate was colourless the decolourisation of the preparation by removing the erythrocytes was a criterion for an adequate perfusion. A further criterion was the vascular resistance of the preparations (PAP, Flow).

A specific examination of the tumour perfusion did not take place because the main focus of the experiments was put on a potential perfusion associated lung edema, i.e. we concentrated on the capillaries of the non-tumourous lung parenchyma.

2.3. Ventilation

The preparations were ventilated with a respirator by suturing the bronchus (or bronchi) end-to-end to a commercially available bronchial tube. A mixture of air and CO2 at room temperature was used as ventilating gas. By adjusting CO2 we could avoid an increase of the pH.

The inspiration–expiration ratio was set at 1:4 to achieve a sufficient expiration volume (the elastic recoil of the thorax is lacking). The tidal volumes were adjusted to the size of the preparations and ranged between 0.1 l and 0.5 l.

2.4. Indicators for edema formation

We used four different parameters for edema formation: (1) weight gain; (2) weight factor: increase of weight relative to the start weight (w − w₀/w₀); (3) disturbances in the kinetics of the alveolar gas exchange; and (4) a histopathological examination of the preparation regarding potential toxic capillary damages as a basis for subsequent lung edema.

2.5. Determination of irinotecan by HPLC in the perfusate and parenchyma

The concentration of irinotecan in all tissue samples was performed by a specialised laboratory (A&M GmbH, Labor für Analytik, Bergheim, Germany) by an assay validated for human plasmas.

The principle of the method was protein precipitation of homogenate samples, followed by high performance liquid chromatography with fluorescence detection. The results of the fluorescence detection could be brought into correlation to the concentration of irinotecan in tissue samples and processed homogenates.

2.6. General procedure

Initially the preparation was perfused for approximately one hour without cytostatic drug in order to exclude a reperfusion edema owing to technical circumsances (mal-positions of the cannuulas, local pulmonary congestion due to venous compression). We would start a dose-escalation schedule infusing irinotecan and continuing the perfusion for up to 3 h if after one hour the parameters indicating a lung edema were still without pathological findings. During this period the edema parameters were checked at regular intervals.

In monotherapy the recommended dose for irinotecan is 350 mg/m² body surface. According to Rivory et al. [11], who proved in their experiments maximum plasma levels for irinotecan of 100 mg/l, we set this concentration as a normal dose. In case of no detectable reperfusion edema after one hour we added the first 100 mg/l. If the parameters for lung edema remained inconspicuous we observed the toxicity of irinotecan at five-, ten-, fifteen- and twentyfold concentration.

3. Results

Our series included five specimens: one upper lobectomy (prep. 3), three lower lobectomies (prep. 1, 4, 5) and one pneumonectomy (prep. 2).

3.1. Increase of weight factor

The increase of weight relative to the start weight (w − w₀/w₀) for each perfusion experiment after a defined period of perfusion is shown in Table 1. All values lying below the ‘critical’ mark of 0.6 illustrate that these preparations developed no significant lung edema. As the chart shows, one value rose up to 1.3 at a tenfold concentration of the drug. But in this case the critical mark had already

<table>
<thead>
<tr>
<th>Prep. 1</th>
<th>0</th>
<th>0.2</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prep. 2</td>
<td>100</td>
<td>0.3</td>
<td>200</td>
</tr>
<tr>
<td>Prep. 3</td>
<td>500</td>
<td>0.1</td>
<td>180</td>
</tr>
<tr>
<td>Prep. 4</td>
<td>0</td>
<td>0.1</td>
<td>80</td>
</tr>
<tr>
<td>Prep. 5</td>
<td>1000</td>
<td>0.8</td>
<td>240</td>
</tr>
<tr>
<td>Prep. 6</td>
<td>1000</td>
<td>1.3</td>
<td>370</td>
</tr>
<tr>
<td>Prep. 7</td>
<td>1500</td>
<td>0.4</td>
<td>180</td>
</tr>
<tr>
<td>Prep. 8</td>
<td>0</td>
<td>0.1</td>
<td>60</td>
</tr>
<tr>
<td>Prep. 9</td>
<td>100</td>
<td>0.2</td>
<td>160</td>
</tr>
<tr>
<td>Prep. 10</td>
<td>2000</td>
<td>0.6</td>
<td>240</td>
</tr>
</tbody>
</table>

Table 1: Increase of net weight in relation to the administered irinotecan dose
been reached before the infusion of the cytostatic drug. We strongly believe that this is due to technical problems during cannulation that could not be explained. Disturbances in gas exchange, however, could not be verified (Table 1).

3.2. Gas exchange

A further indicator for edema formation is the kinetics of alveolar gas exchange. An increase of $pO_2$ in the ventilating gas from $fLO_2=0.21$ to $fLO_2=1$ was followed by an increase of $pO_2$ in the perfusate. The delayed time course and the maximum value of the $pO_2$ change in the perfusate turned out to be a reliable marker for edema formation [10]. The time course of $pO_2$ uptake into the perfusate within the first hour of the experiment could be taken as a calibration curve of the non-edematous lung. After each administration of the drug we determined a further specific gas exchange curve for the preparation. The results in Fig. 1a–e show that the calibration curve and the curve after the administration of the different amounts of irinotecan overlap nearly completely. Thus, an apparent lung edema for all irinotecan concentration up to twenty times higher than the normal concentration could be excluded (Fig. 1).

3.3. Histopathological examination

Tissue samples of non-tumourous parenchyma were taken for histopathological examination to detect potential capillary damage. Pulmonary capillary damage would be associated with chain-reacting processes leading to toxic microvascular injuries and serous-fibrinous exudative reactions in the pulmonary terminal vessels. The histopathological manifestation for these reactions would have been interstitial lung edema, capillary damages and hyaline membranes. None of these manifestations could be verified in any preparation by the pathologist.

3.4. Tissue concentration of irinotecan

Irinotecan concentrations were determined by means of the HPLC technique in tissue samples of tumourous, non-tumourous lung tissue and hilar lymph nodes. It could be shown that the concentrations of tumourous and non-tumourous tissues were within comparable proportions (2.1–26.4 mg/g in non-tumourous tissue vs. 0.06–35.3 mg/g in tumour tissue). Furthermore, the analysis proves an uptake of irinotecan in hilar lymph nodes via lymphatic vessels (Fig. 2).

4. Discussion

The lungs are the organs with the highest predisposition for metastases in carcinoma patients. If the primary location of the carcinoma was controlled, then the metastasectomy can be considered the best curative therapy.
‘The International Registry of Lung Metastases’ could show that metastasectomy is a safe and potentially curative treatment with long-term survival [1].

Non-detectable micrometastases which already existed during the operation are supposed to be the reason for most relapses [3].

The isolated lung perfusion, for the first time depicted by Creech et al. in 1958 [12], is in our judgement an adequate procedure for the above-mentioned unresectable metastases and also an adjuvant option to control the relapses.

This procedure conceives the administration of extreme high doses of cytostatic drugs. Moreover, it enhances the potential efficiency because of no compelling interruption due to dose-related toxic side effects and minimises systemic toxicities.

Already in 1968 Ohno [13] has been able to show that an isolated lung perfusion carried out prophylactically almost doubled the survival rate of certain carcinoma patients although at that time no apparent metastases were detectable.

Currently the most frequently used cytostatic drugs for the isolated lung perfusion are doxorubicin, cisplatin and adriamycin, even though several groups [14, 15] have described independently from each other that these drugs do not have an ideal profile for perfusion: on the one side owing to the high lung parenchyma toxicity and on the other side owing to the low uptake into the tumour tissue in comparison to the non-tumourous parenchyma.

Our experiments show that irinotecan reaches comparable concentrations in tumourous and non-tumourous tissues. Doxorubicin for example, on the other hand, shows a poor uptake into tumour tissue which is documented by a 30-fold lower concentration in the tumour tissue compared to normal lung tissue. Consequently a better anti-tumour effect at comparable drug concentrations in lung tissues and a lower toxicity of irinotecan on non-tumourous tissue can be expected.

Irinotecan in fact has a very interesting pharmacological profile for the isolated lung perfusion because it is not only converted in the metabolical centres like the liver into the much stronger metabolite SN-38 but also in the tumour tissue itself.

In the course of recent studies it advanced to an important cytostatic drug in the treatment of colorectal carcinomas (particularly 5-fluoruracil-(5-FU) refractory), small cell and non-small cell lung cancers (NSCLC, SCLC), ovarian carcinomas and their metastases.

Therefore, the results of our trials provide the basis for phase I studies because they facilitate the application of pharmacologically gained data for the isolated lung perfusion with irinotecan in human beings.

Due to the excellent transferability — contrary to pharmacological or animal experiment — we present much more reliable data to the patients. This also minimises possible side effects, as for example acute severe toxic lung edema. This side effect has been frequently described during phase I trials for the evaluation of the isolated lung perfusion [5, 6].

Despite a dose escalation up to 20 times higher than the normal concentration no significant lung toxicity parameters could be identified.

Both the increase of the net weight and the parameters of the gas exchange remained almost inconspicuous.

In case of toxic lung damage the resulting diffuse alveolar damage would have entailed a respiratory insufficiency or disturbance of gas exchange.

But it also needs to be said that with our above-mentioned method toxic perfusion damages, which develop after hours of perfusion, cannot be analysed. These damages are attributed to special intracorporally mediating systems.

Our series of experiments clearly show an uptake of irinotecan during the isolated perfusions via the pulmonary artery in tumourous tissue as well as in hilar lymph nodes via local lymphatic vessels. Compared to adriamycin, irinotecan is characterised by a favourable proportion of concentration in tumourous and non-tumourous tissue, which may be explained by its high tumour selectivity. This comparably low concentration of irinotecan in non-tumourous tissue will, by analogy with clinical experiences, probably also result in a lower lung toxicity.

References


