Assessment of cisplatin concentration and depth of penetration in human lung tissue after hyperthermic exposure

Michael Rieda,*, Karla Leibleb, Reiner Neuć, Claudius Dieza, Patrick Bednarskid, and Hans-Stefan Hofmann a,d

a Department of Thoracic Surgery, University Medical Center Regensburg, Regensburg, Germany
b Department of Cardiothoracic Surgery, University Medical Center Regensburg, Regensburg, Germany
c Institute of Pharmacy, University of Greifswald, Greifswald, Germany
d Department of Thoracic Surgery, Krankenhaus Barmherzige Brüder Regensburg, Regensburg, Germany

* Corresponding author. Department of Thoracic Surgery, University Medical Center Regensburg, Franz-Josef-Strauß Allee 11, 93053 Regensburg, Germany.
Tel: +49-941-9449810; fax: +49-941-9449811; e-mail: Micha.ried@t-online.de (M. Ried).

Received 25 January 2014; received in revised form 7 April 2014; accepted 16 April 2014

Abstract

OBJECTIVES: The effects of cisplatin on the lung parenchyma during hyperthermic intrathoracic chemotherapy perfusion have not been analysed in detail. The objective of this study was to evaluate both the concentration and depth of the penetration of cisplatin in human lung tissue after hyperthermic exposure under ex vivo conditions.

METHODS: This experimental study was approved by the local ethics committee. Twelve patients underwent pulmonary wedge resections after elective thoracic lobectomies were performed (resected lobe), and the lung tissue (approximately 1–2 cm3) was incubated (in vitro) with cisplatin (0.05 mg/ml; 60 min, 42°C). Subsequent tissue beds (depth, 0.5 mm; median weight, 70–92 mg) were prepared from the outside to the middle, and the amount of cisplatin per tissue weight was analysed using atomic absorption spectrometry. Afterwards, the penetration of cisplatin depth was calculated and related to the different concentrations per tissue.

RESULTS: Cisplatin penetrated into the human lung tissue after ex vivo hyperthermic exposure. The median amount of platinum [nmol cisplatin/g lung tissue] decreased significantly (P ≤ 0.05) depending on the penetration depth: 32 nmol/g (1 mm), 20 nmol/g (2 mm) and 6.8 nmol/g (4 mm). The calculated median concentrations of cisplatin (µg/ml) were 2.4 µg/ml (1 mm), 1.4 µg/ml (2 mm) and 0.5 µg/ml (4 mm), respectively.

CONCLUSIONS: Under ex vivo hyperthermic conditions, cisplatin diffused into human lung tissue. The median penetration depth of the cisplatin was approximately 3–4 mm. The penetration of cisplatin into lung tissue may affect the local therapy of residual tumour cells on the lung surface using hyperthermic intrathoracic chemotherapy perfusion in patients with malignant pleural tumours.

Keywords: Hyperthermic perfusion • Chemotherapy perfusion • Penetration depth • Cisplatin

INTRODUCTION

Despite a multimodality treatment regimen, local tumour recurrence still limits long-term survival in patients with malignant pleural mesothelioma [1, 2]. Radical pleurectomy/decortication and extra pleural pneumonectomy aim to remove all macroscopic tumour lesions [3]. In recent years, the addition of hyperthermic intrathoracic chemotherapy perfusion (HITHOC) after surgical cytoreduction has been performed more frequently to improve local tumour control [4–6]. Immediately after a macroscopic complete tumour resection, residual microscopic tumour lesions can be treated directly with the HITHOC procedure using cisplatin in most patients [7]. An improved outcome and an acceptable incremental morbidity have been reported recently and support the incorporation of HITHOC with cisplatin into multimodality treatment strategies for patients with malignant pleural tumours [8, 9].

Our surgical technique and clinical experience with surgical cytoreduction in combination with HITHOC have been described in previous studies [7]. Despite increasing clinical data in the recent literature, few published data are available on the pharma- cokinetic characteristics of intrapleurally administered cisplatin and its systemic impact [10, 11]. In a previous study, we demonstrated that HITHOC with cisplatin provides high local intrapleural cisplatin concentrations. This potential clinical benefit indicates the pharmacological advantage for the local administration of cisplatin and ensures decreased systemic toxicity [12].

© The Author 2014. Published by Oxford University Press on behalf of the European Association for Cardio-Thoracic Surgery. All rights reserved.
However, only limited data are available concerning the characteristics and impact of cisplatin on the lung parenchyma during the HITHOC procedure. Therefore, the purpose of this study was to determine the concentration of cisplatin and its depth of penetration into human lung tissue after hyperthermic exposure under \textit{ex vivo} and \textit{in vitro} conditions.

**MATERIALS AND METHODS**

**Patients and study design**

This experimental study was approved by the local ethics committee, and written informed consent was obtained from each patient. A total of 12 patients were enrolled in this study. All patients underwent an elective lobectomy via an anterolateral thoracotomy at the Department of Thoracic Surgery, University Medical Center, Regensburg. Afterwards, a pulmonary wedge resection at the periphery of the surgically resected lobe was performed. This wedge resection was performed in all patients using a surgical stapler (GIA™ Universal Stapling System; Covidien Germany GmbH) with an ample safety margin for the pulmonary tumour, when appropriate. The primary end-point of this experimental study was to evaluate whether the chemotherapeutic agent cisplatin diffuses in the lung parenchyma under hyperthermic conditions. Therefore, the detection of cisplatin in human lung tissue was measured using atomic absorption spectrometry (AAS). The secondary end-point was to analyse the depth of the cisplatin penetration into the human lung tissue.

**Experimental setting**

The surgically resected human lung tissue (approximately 1–2 cm$^3$) was incubated \textit{(ex vivo, in vitro)} in a cisplatin solution [0.05 mg/ml in sodium chloride (NaCl) 0.9%] in a microtube (2 ml, Eppendorf, Hauppauge, NY, USA) for 1 h at 42°C. The tissue was rinsed with NaCl, and a tissue block (0.5 cm × 0.5 cm) was punched out spanning from one side to the other side of the tissue block. The punch was embedded in Tissue-Tek® OCT matrix (Sakura, Alphen aan den Rijn, NL), frozen in liquid nitrogen steam and stored at $-80°C$. The frozen punch was serially sliced with a microtome cryostat (Leica CM1900, Nussloch, Germany) from the outside to the inside of the tissue sample. Ten consecutive cryosections (of 50 µm) were collected, filled with NaCl (200 µl/100 mg tissue) and homogenized. For the homogenization, stainless steel beads (diameter, 5 mm; Qiagen, Hilden, Germany) were added and treated for 30–90 s with a TissueLyser II (Qiagen) according to the manufacturer’s instructions. After the addition of an equivalent volume of nitric acid (65%, Merck, Darmstadt, Germany), the suspension was incubated overnight at 80°C in a water bath. After centrifugation (12 000 × g, 30 min, room temperature), the supernatant was transferred into a new microtube and transported to the Institute of Pharmacy at the University of Greifswald for a platinum analysis.

**Platinum analysis**

A Solaar 989 QZ (Unicam) Furnace atomic absorption spectrometer (FAAS) was used to measure the platinum at $\lambda = 265.9$ nm with deuterium lamp compensation as described by Kushev \textit{et al.} \cite{13}. An external standard curve (0.0585–0.585 µg/ml platinum) was used to determine the concentration of the platinum in µg/ml and samples were diluted accordingly with 0.5% HNO$_3$ to give platinum concentrations within the range of the standard curve. The concentrations of platinum in the different tissue layers were determined in triplicate.

**Statistical analysis**

All of the data were collected in a Microsoft Excel spreadsheet for Windows (Microsoft Corp., Redmond, WA, USA). The graphical representation was performed using SigmaPlot 11.0 (Systat Software, Inc., Chicago, IL, USA). SPSS 21.0 software for Windows (SPSS, Inc., Chicago, IL, USA) was additionally used for the statistical analysis. A descriptive analysis was performed, and all values were presented as the median with interquartile ranges (25–75th percentile) when non-normally distributed. The Mann–Whitney U-test was used to compare the different cisplatin concentrations for the non-normally distributed data. A $P$-value of less than 0.05 was assumed to be statistically significant.

**RESULTS**

**Concentration and penetration depth of cisplatin**

Serial tissue layers with a median (25 and 75% percentile) tissue weight of 80 (70/96) mg were analysed for their cisplatin...
concentrations using AAS. The concentration of cisplatin decreased significantly ($P \leq 0.05$) from the interface with the cisplatin solvent to the deeper tissue layers of the lung sample. A regression analysis allowed for the estimation of the concentration as a function of the penetration depth (Fig. 1). For example, at a penetration depth of 2 mm, the median concentration of cisplatin was 1 µg/ml. Figure 2 shows the averaged data for the tissue layers of 1 mm [2.4 µg/ml (depth: 1 mm), 1.4 µg/ml (2 mm), 0.7 µg/ml (3 mm) and 0.5 (4 mm)]. In the deeper tissue layers, the verification of the cisplatin concentrations failed because of the detection limits of the method.

DISCUSSION

Hyperthermic intrathoracic chemotherapy perfusion with cisplatin has become more frequently used in the multimodality treatment of both malignant pleural mesothelioma and pleural thymoma involvement [5, 7]. Beneficial effects of HITHOC regarding the time to local tumour recurrence and overall survival in selected patients has been reported [8, 9]. The present experimental study proved for the first time that, under ex vivo and in vitro hyperthermic conditions, the chemotherapeutic agent cisplatin diffused into human lung tissue. Platinum was still detectable at a depth of 3–4 mm, despite existing visceral pleura on the lung parenchyma. Cisplatin was used at a concentration of 0.05 mg/ml based on prior analyses performed in our working group and our clinical experience with HITHOC [12].

To our knowledge, only a few previous studies have evaluated the pharmacokinetics of intrapleural cisplatin [11, 14]. The benefits of intracavitary chemotherapy are its high local concentration and reduced systemic side effects [10, 15, 16]. Nevertheless, the limiting factor in escalating the intrathoracic cisplatin dose is its systemic toxicity, which can be managed with the appropriate systemic protection, including perioperative hydration and the administration of cytoprotective drugs [12, 17, 18]. In addition, regional hyperthermia is suggested to increase the penetration depth and to enhance the cytotoxicity of the cytostatic drug as well as having its own antineoplastic effects by inducing the potent apoptosis of tumour cells [19, 20]. Morphological and functional investigations showed no damage to the lung parenchyma from the hyperthermic pleural space perfusion with cisplatin [19, 21].

Cisplatin is still the preferred and most commonly used agent for intrathoracic chemotherapy perfusion, either alone or in combination with other cytotoxic drugs. Direct contact of the cytostatic agent with the residual tumour cells allows the penetration of the cancer cells by simple diffusion [22]. However, the penetration may be limited to a few millimetres, emphasizing the importance of a preferably macroscopic complete resection and a multimodality treatment [12, 14].

In a recent in vitro evaluation of Cameron et al., three human mesothelioma cell lines showed no particular sensitivity to heat, but did show a dose–response relationship for cisplatin at 42°C. Cell viability decreased with increasing doses of cisplatin (1, 2 and 4 µg/ml) and with time. Finally, most of the reduction was attributable to chemotherapy and not to the hyperthermia of 42°C [23]. In addition, another study characterized the cisplatin chemosensitivity of malignant mesothelioma cell lines and the dose-dependent cisplatin-induced cell death associated with apoptosis. The percentage of viable mesothelioma cells was approximately 60–80% at a cisplatin concentration of 1 µg/ml [24]. In both of the experimental investigations, the concentrations of cisplatin, which caused significant reductions in the viable mesothelioma cells, were nearly comparable with the concentrations of cisplatin measured in our samples of human lung tissue. The concentration of cisplatin decreased from 2.4 µg/ml at a penetration depth of 1 mm to approximately 0.5 µg/ml at a depth of 4 mm in the lung tissue. Accordingly, the local cisplatin concentrations on the lung surface and lung parenchyma may influence local tumour control in mesothelioma patients after macroscopic complete surgical cyto-reductions.

In an actual clinical situation, the HITHOC procedure is performed after a radical pleurectomy and decortication. However, there may be areas on the lung surface with an intact visceral pleura as well as microscopic tumour lesions. The HITHOC procedure should reach these areas in particular and treat suspicious tumour cells despite leaving the visceral pleura intact. This is one of the proposed advantages of HITHOC. Based on these results, we now know that cisplatin penetrates into the lung tissue, despite an intact visceral pleura. Consequently, we also need an effective local chemotherapy for these areas, which cannot be completely decorticated.

In conclusion, the cisplatin that diffuses into the lung tissue may induce apoptosis of the residual tumour cells. However, the effective concentration in the lung tissue under in vivo conditions remains unclear. Nevertheless, the penetration of cisplatin into the lung tissues may improve the local therapy of residual microscopic tumour cells on the lung surface with the use of hyperthermic intrathoracic chemotherapy perfusion in patients with malignant pleural tumours after lung-sparing radical tumour resections.

Study strengths and limitations

In this experimental study, the extent of local cisplatin penetration in human lung parenchyma was investigated under ex vivo and in vitro conditions using AAS. In this ex vivo study, only the lung tissue with intact visceral pleura was used. Thus, the concentrations of cisplatin in decorticated lung tissue might be higher than shown here. Furthermore, the pharmacokinetics of perfused living lung tissue differs substantially from the pharmacokinetics observed in an ex vivo model. This study was only a further step in providing more data to support and warrant the clinical applications of HITHOC. Further clinical and pharmacological investigations with respect to the impact of various chemotherapeutic drugs and their systemic influence with regard to lung tissue are strongly needed. Only with more evidential data from basic science can the optimal clinical application of HITHOC and chemotherapeutic agents be validated.

ACKNOWLEDGEMENTS

The authors appreciate the technical assistance of K. Bielenberg, Department of Cardiothoracic Surgery, University Medical Center, Regensburg.
Conflict of interest: none declared.

REFERENCES


