Intrathoracic polymeric films containing cisplatin for malignant pleural mesothelioma in a rat tumour model: a preliminary study

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Abstract

Objective: This study aims to investigate the effect of intrathoracic polymeric films containing cisplatin on the local recurrence of malignant pleural mesothelioma in a rat tumour model. Methods: An orthotopic rat recurrence model of malignant pleural mesothelioma was used. Five animals per group were evaluated. Polymeric films (4.5 cm diameter) for the local delivery of anticancer drug were constructed: hyaluronate, chitosan and the combined dual-layer polymers were loaded with cisplatin at a concentration of 100 mg m−2. Animals without any adjuvant therapy were used as control. Mesothelioma cells were injected subpleurally in the anaesthetised rats. Six days later, a pleural tumour of 5.5 mm was resected and a left pneumonectomy and pleural abrasion were performed. Thereafter, the cisplatin-loaded and unloaded films or cisplatin solution were intrapleurally applied, according to randomisation. After 6 days, animals were euthanised and organs harvested for morphological and histological evaluations. The primary endpoint was the volume of tumour recurrence. The secondary endpoints were treatment-related toxicity; cisplatin serum concentration evaluated at different time points; and cisplatin concentration in the pleura measured at autopsy. Analysis of variance (ANOVA) was used for statistical analysis. Bonferroni correction was applied for comparison between all groups. Results: Tumour volume was significantly reduced in the hyaluronate cisplatin and hyaluronate–chitosan cisplatin groups in comparison to control groups (p = 0.001 and p < 0.0001, respectively). Animals treated with hyaluronate–chitosan cisplatin had a tumour recurrence significantly lesser than animals treated with cisplatin solution (p = 0.003) and hyaluronate cisplatin (p = 0.032). No toxicity related to the different treatments was observed. On postoperative days 1 and 2, cisplatin was detected in the serum at a concentration six- and sevenfold significantly higher in the hyaluronate cisplatin and hyaluronate–chitosan cisplatin groups, in comparison to cisplatin solution, and was maintained over time. Cisplatin levels in the pleura were higher in the hyaluronate–chitosan cisplatin group than in all others. Conclusions: Hyaluronate–chitosan cisplatin was significantly effective in reducing tumour recurrence compared with cisplatin solution. Hyaluronate and hyaluronate–chitosan loaded with cisplatin assured significantly higher and more prolonged plasmatic drug concentrations than cisplatin solution without increasing toxicity.

Keywords: Mesothelioma; Intrapleural; Cisplatin; Hyaluronate; Chitosan; Chemotherapy

1. Introduction

The annual report on the current status of cancer burden in the United States revealed an overall decrease in the incidence of cancer and death rates from all cancers combined in men and women [1]. In Italy, a recent analysis of cancer mortality demonstrated an overall favourable pattern over recent years [2].

On the other hand, the incidence of malignant pleural mesothelioma (MPM) is increasing throughout the world, and it is expected to peak around the next 10–20 years [3]. In Japan, there are expected to be about 100 000 deaths due to MPM during the next 40 years [3]. Further, in Italy, the incidence and mortality trends for MPM are still rising [2]. This course is due to the long latent period of 20–40 years from the time of asbestos exposure, its main risk factor, until tumour development and growth [3].

Median survival of mesothelioma patients is still poor despite different treatment strategies, with long-term survivors seen only after multimodality programmes [4,5]. Distant metastases have rarely occurred in MPM; however, it can infiltrate the chest wall, diaphragm and mediastinal...
structures. Local tumour recurrence determines a patient’s bad prognosis and represents the real challenge related to MPM. Based on this noteworthy concept and on the fact that intravenous chemotherapy is not sufficiently effective in controlling tumour expansion, new loco-regional treatment options have been evaluated taking into consideration that systemic side effects could be potentially reduced [6].

Multimodal approaches encompassing intrapleural therapy have been demonstrating encouraging results in human and experimental settings [6,7]. Recently, the intracavitary local application of chemotherapy and immunotherapy for the treatment of malignant pleural mesothelioma has been reported in a well-established animal model [8]. The advantages of using this rat mesothelioma model are the following: orthotopic tumour growth, very high reproducibility, and optimal study of local recurrence in standardised conditions [6]. Opitz et al. were able to demonstrate a better tumour control when cisplatin, intrapleurally applied, was loaded onto a fibrin sealant called Vivostat. In that manner, the drug could stay longer in contact with the tumour and prolong the anti-tumour effect [6].

Based on these results, polymeric devices in the form of thin films were constructed with the aim of increasing the local concentration of the chemotherapeutic drug and of achieving a long-lasting cytotoxic effect. Hyaluronate and chitosan are polysaccharidic polymers that have been studied for drug-delivery applications because of their interesting biopharmaceutical properties. Hyaluronate (hyaluronan or hyaluronic acid) consists of repeating disaccharide units of -glucuronic acid and (1→3)-N-acetyl-D-glucosamine [9]. It is widely distributed in mammalian cells and tissues, but it is primarily found in the synovial fluid, vitreous humour of the eye and dermis. It is also found in bacteria such as staphylococci and streptococci; these have been biotechnologically developed and now are a large source of commercial hyaluronate for pharmaceutical and medical applications [9]. Hyaluronate plays a fundamental role in the organisation and structure of the extracellular matrix, the regulation of cell adhesions and morphogenesis [9]. Since hyaluronate is native to the body, it is non-immunogenic and could be an ideal material for drug delivery.

Hyaluronate has been used in different pharmaceutical forms, such as microspheres, hydrogels, polymer conjugate, dry powder and implants [9]. Rosato et al. performed in vitro and in vivo studies using the combination paclitaxel—hyaluronate for the intracavitary treatment of superficial bladder cancer [10]. They observed significantly improved results in comparison to conventional paclitaxel in terms of hydrosolubility, in vitro activity against human bladder cancer cells and in vivo biocompatibility.

Chitosan is a natural and immunogenic polymer with mucoadhesive properties that can be used to enhance the therapeautic efficacity of a drug, either by increasing the contact time at the site of action or by enhancing the permeation through mucosal barriers by opening tight junctions [11]. Its potential roles in drug-delivery systems have been well described [12]. Jauhari and Dash, for example, developed a chitosan in situ gel delivery system that could sustain the release of paclitaxel over time [12]. The aim of this preliminary study is to test the effect of the intrapleural application of hyaluronate- and chitosan-based films loaded with cisplatin on the reduction of tumour volume in a rat model of malignant pleural mesothelioma.

Secondary endpoints were the following: the treatment-related toxicity measured by the analysis of the animal’s blood at different time points and histological examination of rat organs removed during the autopsy, and cisplatin determination in the serum and in pleural samples.

2. Materials and methods

The study protocol was approved by the local veterinary committee on 31 March 2008 and the experiments were performed according to the Guiding Principles in the Care and Use of animals (DL 116/92).

2.1. Animal and tumour cell line

The rat model of malignant pleural mesothelioma used in the experiment was first reported by Kucharzczuk in 1995 and extensively studied by the laboratory of thoracic surgery of the University Hospital of Zurich [6,8,13]. Male Fisher rats 344 weighing 280–300 g (mean 291 g) were purchased from Charles River Laboratories (23885 Calco, LC, Italy). Upon arrival, the animals were placed in the animal facility of the Department of Pharmacology of the University of Parma. They were kept on a schedule of 12 h of light/12 h of darkness; food and water were supplied ad libitum.

IL-45 was the mesothelioma cell line used in the experiment. The cells were kindly donated by the laboratory of the University Hospital of Zurich (Zurich, Switzerland). The tumour cell line originally derived from asbestos-induced peritoneal mesothelioma in rats [13].

The tumour cells were cultured in RPMI medium supplemented with glutamine 2 mM, 10% foetal calf serum, 100 U ml\(^{-1}\) penicillin and 100 mg ml\(^{-1}\) streptomycin, and placed in a 37°C, 5% CO\(_2\) incubator.

Before the experiment, 1 million cells were counted and re-suspended in 50 μl of complete growth medium and the tubes were put on ice.

2.2. Drug and polymeric films

The anticancer drug, cisplatin, with purity higher than 99.9%, was purchased from Sigma–Aldrich (Saint Louis, USA). Chitosan was purchased from Primex (ChitoClear®, Siglufjordur, Island). Hyaluronic acid (Ophthalmic HA, code 811240) was a kind gift of Fidia Pharmaceuticals (Abano Terme, PD, Italy). Other reagents and excipients were purchased from ACEF (Fiorenzuola d’Arda, PC, Italy).

Two types of film were produced, one containing chitosan and the other containing hyaluronic acid as the main component (Fig. 1). Polyvinyl alcohol, polyethylene glycol and sorbitol were used as film-forming and plasticiser agents.

The polymeric films were produced by lamination (2 mm layering, variable opening blade Mytutoyo, Italy) of the viscous solution containing the drug and all the excipients (total components content in solution 8%, w/v), followed by drying (55°C, 6 h).
Beside cisplatin-loaded films, blank films were produced for comparison. Cisplatin-loaded films contained 0.5% (w/w) of drug on dry weight.

Combined films were obtained by simply putting the two different polymeric films in contact for 10–15 min under the pressure of a weight of 10 kg. The two films, being slightly adhesive, stick together and can be cut together.

For in vivo experiments, film discs of 4.5 cm diameter were cut and placed in a thermosealed packaging until used. The same dosage of cisplatin (approximately 0.9 mg) was present in a 1.5-ml NaCl 0.9% solution and intrapleurally administered in the animals belonging to the cisplatin solution treatment group.

Films were characterised for their thickness and their weight per square centimetre was also calculated. Drug release was determined in vitro in physiologic solution (NaCl 0.9%); cisplatin concentration was determined by high-performance liquid chromatography (HPLC) (Pharm. Eur. 6.0).

In vitro studies were extensively performed to characterise the mechanical properties of the films from a chemical and physical point of view. Concerning in vitro release, a complete, more specific study of the formulation is reported elsewhere (data submitted). The tumour cells (IL-45) were also incubated with the different polymeric films for evaluating the cytotoxic effect (IC50) (data submitted).

2.3. Treatment groups

Thirty-five rats were randomly assigned to the different treatment groups named: (a) control (no adjuvant therapy after the surgical procedure), (b) chitosan, (c) hyaluronate, (d) cisplatin solution (1.5 ml NaCl 0.9% containing cisplatin at a concentration of 0.6 mg ml⁻¹ to have a dose of 3 mg kg⁻¹ or 100 mg m⁻²), (e) chitosan cisplatin, (f) hyaluronate cisplatin and (g) hyaluronate–chitosan cisplatin (combined polymeric film). The randomisation was performed prior to tumour cell inoculation by means of random numbers sequence.

2.4. Surgical procedure and polymeric film application

The surgical procedures have already been reported in detail [6]. Briefly, a tumour nodule of 5.5 mm mean diameter (range: 4.4–6.1 mm) always developed 6 days after subpleural mesothelioma cell inoculation. After pleural tumour resection and pneumonectomy were carried out, animals received the adjuvant treatment according to randomisation. The films were removed from their packaging and were accurately placed into the cavity to cover the entire pleural surface. The application of the device over the entire chest cavity was facilitated by a small cotton tip. In all the cases, the film was well adherent to the parietal pleura at the end of the procedure.

2.5. Cisplatin determination in the serum and pleura

Cisplatin determination in the serum and pleura was performed blindly by CM. All the samples were unfrozen just before starting the procedure. Experimental measurements were made on the ICP-MS X Series II (ThermoFisher Corporation, Waltham, MA, USA) equipped with an AS-500 autosampler (CETAC, Omaha, NE, USA) operating under XRF interface standard conditions. The operating parameters of the ICP-MS instrument were as follows: RF power 1400 W, coolant gas flow 15.5 l min⁻¹, auxiliary gas flow 0.98 l min⁻¹, nebuliser gas flow 0.87 l min⁻¹, nickel standard X1 cones, peak jumping data acquisition mode, dwell time 100 ms, duration time 60 s and standard resolution. The instrument optimisation was performed daily with the auto-tune procedure to assure a response of at least 80 000 cps/µg l⁻¹ for indium and 100 000 cps/µg l⁻¹ for uranium in the high mass range. All the platinum isotopes were acquired to account for spectroscopic interferences, but only 195Pt was used for calculations. All the analysed samples were previously treated with aqua regia in a microwave-assisted MLS-1200 MEGA (Milestone, Sorisole, Italy) apparatus equipped with an MDR-1000-6 rotor and diluted with ultrapure water to a final volume of 10 ml.

2.6. Blood samples and examination

Blood samples were collected at days 0, 1, 3 and 6 (day of the autopsy of the animals). The rat was placed under the hood and put in an apposite box for ether anaesthesia induction. Once the rat was anaesthetised, it was positioned in a supine position and blood was obtained through a puncture of one of the veins of the tongue. A total of 1.5 ml of blood was collected in a plastic micro-container. Half a millilitre was used for standard blood test; 1 ml was centrifuged for 10 min at 1000 × g and the serum was tested for renal and hepatic parameters. Fifty microlitres of serum were separated and stored at –80 °C until analysis of cisplatin determination. After the procedure, the rats were injected subcutaneously with 2 ml physiologic solution and observed for some minutes in their cages.

Haematological analysis was performed by electronic cell counter HEMA 5 (SEAC, Italy) using sterile tubes with the addition of lithium heparin as anticoagulant at a concentration of 35 UI heparin per millilitre blood (Sarstedt, Numbrecht, Germany). Electronic count of blood cells was performed blindly by CM. All the samples were unfrozen just before starting the procedure. Experimental measurements were made on the ICP-MS X Series II (ThermoFisher Corporation, Waltham, MA, USA) equipped with an AS-500 autosampler (CETAC, Omaha, NE, USA) operating under XRF interface standard conditions. The operating parameters of the ICP-MS instrument were as follows: RF power 1400 W, coolant gas flow 15.5 l min⁻¹, auxiliary gas flow 0.98 l min⁻¹, nebuliser gas flow 0.87 l min⁻¹, nickel standard X1 cones, peak jumping data acquisition mode, dwell time 100 ms, duration time 60 s and standard resolution. The instrument optimisation was performed daily with the auto-tune procedure to assure a response of at least 80 000 cps/µg l⁻¹ for indium and 100 000 cps/µg l⁻¹ for uranium in the high mass range. All the platinum isotopes were acquired to account for spectroscopic interferences, but only 195Pt was used for calculations. All the analysed samples were previously treated with aqua regia in a microwave-assisted MLS-1200 MEGA (Milestone, Sorisole, Italy) apparatus equipped with an MDR-1000-6 rotor and diluted with ultrapure water to a final volume of 10 ml.

2.7. Sample preparation for histological examination

Six days after the treatment (surgical resection and intrapleural therapy), all the animals were humanely killed by carbon dioxide inhalation. For histological examination, each lung was isolated and cut into slabs of 1 cm thickness. Slabs were fixed in a 10% formalin solution for 7 days. After fixation, the slabs were dehydrated in ascending grades of ethanol and embedded in paraffin, before being cut into 4-μm-thick sections. Sections were stained with haematoxylin and eosin (H&E) and examined under a light microscope (Olympus BX51, Japan).
euthanised and subsequently necropsy was performed. Chest wall, kidneys, liver, heart and right lung were taken and fixed for at least 2 days in 4% neutral buffered formalin solution. The length, width and thickness of the tumour nodule were measured and the volume of the recurrence evaluated using the formula: \( V = \frac{4}{3} \pi abc \) (\( a, b, c \) = semi-axes of the tumour). Each specimen was cut into 4-mm slices, dehydrated and paraffin embedded. Slices of 5-μm thickness were put on a Superfrost slide and stained with haematoxylin and eosin (HE). The kidneys were also evaluated using a Periodic Acid Schiff (PAS) stain for histochemical investigation. Chest walls, after formalin fixation, were cut into various slices depending on the location of the tumour.

2.8. Statistics

The Statistical Package for the Social Science software for Windows (SPSS 17.0, Chicago, IL, USA) was used for statistical analysis. Data are given in mean and standard deviation. Analysis of variance (ANOVA) was performed to compare all the groups. Bonferroni correction was applied for comparison between all groups. \( P \) values < 0.05 were considered to be significant.

3. Results

3.1. Polymeric film characterisation

All polymeric films produced were flexible and resistant, providing a good material for handling during surgical application. Chitosan films were more resistant, while hylauronic acid-based films were more plastic. Cisplatin loading had no relevant effect on the mechanical properties of hyaluronate films. In any case, films were easily cut into the desired shape, and handling and application on the chest wall during surgery were simple and satisfactory.

Concerning in vitro release, a complete and more specific study of the formulation is reported elsewhere. Briefly, the drug was released more rapidly by hyaluronate films, with an almost complete release of cisplatin in 72 h. This behaviour was due to the high water solubility of hyaluronate polymers. By contrast, chitosan was insoluble in aqueous medium and chitosan-based films evidenced a much longer release with only a small fraction of the drug released after 30 days.

3.1.1. Animal experiment

Five animals per group were studied. All the animals tolerated the surgical procedures and treatments well. No intra-operative death was registered. Four rats died within the second postoperative day; all dead animals were replaced by other rats. No analgesic drugs were injected after the first postoperative day.

Histological analysis of the tumours invading the chest wall showed a sarcomatoid malignant pleural mesothelioma. Tumour measurements are detailed in Table 1. Animals treated with hyaluronate—chitosan cisplatin had tumours of significantly smaller length and width than animals receiving cisplatin solution as adjuvant treatment (\( p = 0.009 \)) and \( p = 0.005 \), respectively). Control animals developed a larger tumour recurrence in comparison to animals treated with cisplatin (Fig. 2). Specifically, tumour volume was significantly lower in animals treated with hyaluronate—chitosan cisplatin in comparison to controls (\( p < 0.0001 \)), cisplatin solution (\( p = 0.003 \)) and animals treated with hyaluronate cisplatin (\( p = 0.032 \)) (Fig. 3). Hyaluronate—cisplatin was as effective as cisplatin solution in reducing tumour recurrence. Chitosan film loaded with cisplatin was not effective in reducing tumour recurrence when compared to cisplatin solution. The results of this study are shown in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Control</th>
<th>Chitosan</th>
<th>Hyaluronate</th>
<th>Cisplatin solution</th>
<th>Hyaluronate cisplatin</th>
<th>Chitosan cisplatin</th>
<th>Hyaluronate—chitosan cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (mm)</td>
<td>21.7 ± 2.9</td>
<td>17.7 ± 4.1</td>
<td>19.3 ± 5.7</td>
<td>5.5 ± 3.4</td>
<td>2.75 ± 2.1</td>
<td>20.8 ± 3.8</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>27.7 ± 2.5</td>
<td>24.3 ± 6.1</td>
<td>23.5 ± 9.2</td>
<td>5.2 ± 3.1</td>
<td>2.5 ± 2.9</td>
<td>21 ± 2.9</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>7 ± 6.1</td>
<td>3.9 ± 1.1</td>
<td>6.1 ± 3.1</td>
<td>2.1 ± 1.0</td>
<td>1.8 ± 1.7</td>
<td>5.3 ± 3.3</td>
</tr>
</tbody>
</table>

ANOVA was used and Bonferroni correction was applied. Length: hyaluronate—chitosan cisplatin versus control groups, \( p < 0.0001 \); hyaluronate—chitosan cisplatin versus cisplatin solution, \( p = 0.009 \). Width: hyaluronate—chitosan cisplatin versus control groups, \( p < 0.0001 \); hyaluronate—chitosan cisplatin versus cisplatin solution, \( p = 0.005 \). Thickness: hyaluronate—chitosan cisplatin versus control, \( p = 0.026 \). Data are given in mean and standard deviation.

Fig. 2. Control (A–B) versus hyaluronate—chitosan cisplatin (C–D). (A) Macroscopic appearance of a large tumour recurrence 6 days after tumour and lung resection without adjuvant treatment. (B) Microscopic view (HE × 2): the tumour has uncontrolled extension and infiltrates the periosteum of the rib (chest wall on the left; pleural cavity on the right). (C) Macroscopic appearance of the chest wall 6 days after tumour and lung resection and intrapleural application of hyaluronate—chitosan cisplatin: no macroscopic tumour is visible. (D) Microscopic view (HE × 4): no tumour growth is visible; the inner surface of the chest wall is coated with a sheet from a fibrinous inflammatory reaction. HE: haematoxylin—eosin stain; F: fibrin-inflammatory reaction; M: intercostal muscle; R: rib; T: tumour.
Although a moderate cisplatin-related toxicity was observed in the animals treated with cisplatin irrespective of the different polymeric films, the difference was not statistically significant. Data are given in mean and standard deviation.

3.1.2. Histological examination

The epithelial cells of proximal tubules of all rats of groups a (control), b (chitosan) and c (hyaluronate) were slightly swollen, with moderate vacuolar degeneration and pyknosis of nuclei. The same lesions were slightly increased in the rats of group e (chitosan–cisplatin) and markedly increased in all rats of groups d (cisplatin solution, f (hyaluronate cisplatin) and g (chitosan–hyaluronate cisplatin), although the differences did not reach statistical significance (p = 0.08). Tubules of the medulla and the papilla of all rats treated with cisplatin (groups d, e, f and g) were filled with homogeneous eosinophilic material (hyaline casts). Rare tubules containing the same material were observed in a few rats of groups b and c. Tubules lined by basophilic epithelial cells with a plump vesicular nuclei with prominent nucleoli (tubular regeneration) and tubular ectasia were observed in groups d and g (cisplatin solution and combined film). Tubular regeneration and tubular ectasia were very mild in groups c and f and absent in the other groups. Moderate to severe tubular necrosis, with occasional tubular mineralisation, was present only in the rats of groups d, f and g (cisplatin-based treatment) and it was not statistically different (p = 0.09).

Histological exams of liver revealed a mild focal necrosis irrespective of the different treatments administered.

3.1.3. Cisplatin levels

On postoperative days 1 and 2, cisplatin was detected in the serum at a concentration six- and sevenfold higher in the animals treated with hyaluronate cisplatin and hyaluronate–chitosan cisplatin, respectively, in comparison to those different (p = 0.9 and p = 1.0, respectively). In the animals treated with cisplatin, no significant toxicity related to the two different polymers was noted.

Transaminases were also considered (Table 2); irrespective of the different treatments, we observed a peak of serum levels 24 h after the surgical procedure and we could not detect a statistically significant difference within all groups (p = 0.9); thereafter, the concentration gradually decreased to baseline value.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Chitosan</th>
<th>Hyaluronate</th>
<th>Cisplatin solution</th>
<th>Hyaluronate cisplatin</th>
<th>Chitosan cisplatin</th>
<th>Hyaluronate–chitosan cisplatin</th>
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<tbody>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>0.9 ± 0.5</td>
<td>0.9 ± 0.2</td>
<td>1.2 ± 0.9</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.5</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.2 ± 0.4</td>
<td>0.9 ± 0.9</td>
<td>1.2 ± 0.8</td>
<td>1.1 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.4 ± 0.5</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Day 3</td>
<td>1.0 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>1.1 ± 0.9</td>
<td>1.3 ± 0.9</td>
<td>0.8 ± 0.3</td>
<td>0.7 ± 0.9</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>Day 6</td>
<td>1.0 ± 0.3</td>
<td>1.4 ± 0.5</td>
<td>2.4 ± 1.1</td>
<td>3.2 ± 1.4</td>
<td>5.6 ± 0.5</td>
<td>1.4 ± 0.5</td>
<td>5.3 ± 0.7</td>
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<tr>
<td><strong>Urea (mg/dl)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>50.6 ± 5.5</td>
<td>47.0 ± 7.2</td>
<td>52.3 ± 3.9</td>
<td>50.9 ± 2.3</td>
<td>50.5 ± 3.3</td>
<td>48.4 ± 1.5</td>
<td>48.1 ± 1.2</td>
</tr>
<tr>
<td>Day 1</td>
<td>46.5 ± 3.4</td>
<td>31.7 ± 4.9</td>
<td>49.7 ± 2.8</td>
<td>57.8 ± 4.2</td>
<td>56.9 ± 2.2</td>
<td>88.4 ± 2.5</td>
<td>115.1 ± 9.4</td>
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<tr>
<td>Day 3</td>
<td>40.0 ± 6.4</td>
<td>39.6 ± 3.4</td>
<td>44.5 ± 1.9</td>
<td>139.9 ± 5.9</td>
<td>40.6 ± 2.3</td>
<td>44.8 ± 2.9</td>
<td>161.7 ± 8.0</td>
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<tr>
<td>Day 6</td>
<td>41.0 ± 5.3</td>
<td>44.7 ± 4.5</td>
<td>49.8 ± 2.1</td>
<td>344.1 ± 4.4</td>
<td>408.3 ± 6.2</td>
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<td>305.7 ± 3.4</td>
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<td><strong>GOT (U/L)</strong></td>
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<tr>
<td>Baseline</td>
<td>52.4 ± 5.5</td>
<td>56.9 ± 7.2</td>
<td>68.0 ± 3.9</td>
<td>71.0 ± 4.3</td>
<td>73.7 ± 2.3</td>
<td>56.1 ± 0.9</td>
<td>76.9 ± 1.9</td>
</tr>
<tr>
<td>Day 1</td>
<td>181.0 ± 3.4</td>
<td>212.0 ± 2.9</td>
<td>65.7 ± 1.8</td>
<td>171.7 ± 2.2</td>
<td>262.0 ± 4.2</td>
<td>248.1 ± 4.5</td>
<td>340.1 ± 3.4</td>
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<tr>
<td>Day 3</td>
<td>53.9 ± 4.4</td>
<td>111.0 ± 8.4</td>
<td>59.1 ± 1.9</td>
<td>95.1 ± 8.9</td>
<td>82.3 ± 5.3</td>
<td>58.5 ± 2.5</td>
<td>212.7 ± 6.0</td>
</tr>
<tr>
<td>Day 6</td>
<td>61.0 ± 4.3</td>
<td>45.5 ± 4.5</td>
<td>78.8 ± 3.1</td>
<td>89.0 ± 4.4</td>
<td>86.9 ± 4.2</td>
<td>52.7 ± 4.1</td>
<td>84.4 ± 4.1</td>
</tr>
</tbody>
</table>

Although a moderate cisplatin-related toxicity was observed in the animals treated with cisplatin irrespective of the different polymeric films, the difference was not statistically significant.
treated with cisplatin solution (Fig. 4). This difference was maintained over time: at autopsy, it was 508.25 ng ml⁻¹ for hyaluronate—chitosan cisplatin and 507.5 ng ml⁻¹ for hyaluronate—chitosan cisplatin in comparison to 72.9 ng ml⁻¹ for the cisplatin solution group ($p = 0.032$ and $p = 0.030$, respectively). The elevated plasma dosage of cisplatin did not result in a major systemic toxicity. The differences reached a statistical significance as shown in Fig. 4.

Cisplatin level was also measured in parietal pleural samples. The level of cisplatin detected was very high for hyaluronate—chitosan cisplatin in comparison to all other groups (Fig. 5): in particular, we detected 83.1 μg g⁻¹ (micrograms per gram of tissue) in pleural samples of rats receiving hyaluronate—chitosan cisplatin compared with 14.5 μg g⁻¹ and 4.14 μg g⁻¹ in rats treated with cisplatin solution and hyaluronate cisplatin, respectively. The difference was not statistically different.

4. Discussion

In a multimodal setting, intrapleural therapy for MPM has a solid rationale, although new approaches are necessary in terms of drug-delivery system and mode of delivery. In our experiment, we were able to achieve an optimal tumour response maintaining very high local and plasmatic cisplatin concentrations without increasing systemic toxicity, using innovative polymeric films for controlled drug release.

To our knowledge, Eiselsberg was the first to propose radical surgery for MPM, in Vienna in 1922. But it was not until the late 1950s and 1960s that the first small series of patients began to appear: although some authors reported the usefulness of pleuropneumonectomy in patients with MPM, poor long-term survival results necessitated a reappraisal of the role of pleuropneumonectomy [14].

In 1979, Butchart et al. compared the results of pleuropneumonectomy in 29 patients and the results of non-surgical treatments in 17 patients with pleural mesothelioma [15]. They correlated the occurrence of postoperative complications and survival with tumour stage and histological subtype and concluded that pleuropneumonectomy could not be justified as the treatment of choice in all cases of MPM but that only early-stage epithelial cases should be subjected to pleuropneumonectomy.

In the late 1970s, it was suggested that when surgery was supplemented by radiotherapy or chemotherapy, there was an improvement in the overall survival [16]. At that time, Aisner and Wiernik argued that a desirable avenue of approach in the treatment of MPM would be to combine a localised form of therapy with a systemic approach. Many studies in animals showed that the combination of different methods of therapy was superior to the single application of these treatments [16].

Considering the results of systemic chemotherapy and that MPM tends to remain confined to the pleural space for much of its clinical course, during the 1980s, there was an increasing interest in intracavitary chemotherapy in patients with peritoneal and pleural mesothelioma [17]. Cisplatin-based intrapleural chemotherapy became an increasingly accepted treatment option for patients with malignant pleural disease [18,19].

In 1991, Sugarbaker et al. proposed a multimodality protocol including extrapleural pneumonectomy and adjuvant chemotherapy with or without radiotherapy [20]. The authors reported an acceptable rate of postoperative morbidity and mortality, and the survival rates were 70% at 1 year and 48% at 2 years. This series was then adjourned in 1993 with an improvement in operative mortality.

Lerza et al. investigated the combination of cisplatin (60 mg m⁻²) and carboplatin (270 mg m⁻²) administered intrapleurally in patients with malignant pleural effusion [19]. Mean concentrations measured in the pleural cavity and plasma were 717 μg ml⁻¹ and 5.1 μg ml⁻¹, respectively. Taking into account the increased dosage of chemotherapeutic drugs and the different time points of measurements described by Lerza, we could argue that our results are similar in terms of cisplatin detection.

In 1999, Ratto et al. performed a pharmacokinetic study with the aim of evaluating the effect of the extent of resection (pleuropneumonectomy or pleurectomy/decortication)
combined with hyperthermic pleural space perfusion with cisplatin in patients with MPM [21]. Cisplatin was administered at a concentration of 100 mg m\(^{-2}\) for 1 h: the levels of cisplatin found locally (lung tissue and endothoracic fascia) ranged from 3.96 \(\mu\)g g\(^{-1}\) to 5.25 \(\mu\)g g\(^{-1}\), which were very low compared with our data. Although we did not look at the rats in very first hours after the intrapleural treatment, considering the pharmacokinetics of the cisplatin solution group (Fig. 4), we can assume that the real peak of platinum concentrations would be very early, as previously reported [13].

As we observed in our experiment, significant plasmatic concentrations of cisplatin were also found by Ratto et al. [21] In agreement with Rusch et al. [18] they described that after pleuropneumonectomy, cisplatin was rapidly absorbed from the pleural space, reaching plasmatic concentrations similar to those obtained after intravenous infusion.

In the past 10 years, several studies have been published evaluating different clinical characteristics (age, stage and histology) and therapy options correlated to patients’ survival; only multimodal approaches employing extrapleural pneumonectomy and adjuvant chemo- and radiotherapy have shown prolonged survival data [22—24].

More recently, Krug et al. reported the results of 77 patients with MPM who underwent trimodality therapy encompassing four cycles of pemetrexed (500 mg m\(^{-2}\)) plus cisplatin (75 mg m\(^{-2}\)), followed by extrapleural pneumonectomy and radiotherapy (54 Gy) [4]. The 40 patients who completed the entire therapy had a median survival of 29.1 months and a 2-year survival rate of 61.2%; 40% of the patients had shown a recurrence within 18 months. A similar recurrence rate was observed by de Perrot et al. in 30 patients after neo-adjuvant chemotherapy, extrapleural pneumonectomy and hemithoracic radiation: in N\(_0\) patients, median survival was 59 months [5].

Based on these data, the combination of surgery and neo-adjuvant or adjuvant treatment is currently considered an appropriate option for patients with good performance status. In any case, taking into consideration the very high percentage of local tumour relapse, new treatment options needed to be assessed.

A major issue in studies evaluating intracavitary therapy with cytotoxic drugs for the treatment of locally invading cancers is the resulting systemic drug exposure. In our study, the intrapleural administration of cisplatin loaded onto hyaluronate and hyaluronate—chitosan resulted in significantly prolonged plasma levels of the cytotoxic drug in comparison to cisplatin solution (Fig. 4). It is therefore likely that the pharmacokinetic of the drug, combined with its controlled release from the polymeric film, influenced the exposure of the tumour to cisplatin locally and systemically. The highest levels of platinum compounds that we detected intrapleurally (mean 83.1 \(\mu\)g g\(^{-1}\)) were about 1000 times as high as platinum concentrations found systemically (mean 507.5 ng ml\(^{-1}\)). Although we measured very elevated plasmatic drug concentrations, significant systemic toxicity has been not observed. Considering the levels of creatinine at day 6 (Table 2) after administration of hyaluronate cisplatin (95% CI 0.0—0.51) and hyaluronate—chitosan cisplatin (95% CI 0.0—0.78), clinically relevant differences could be excluded.

In a study evaluating the intrapleural application of cisplatin with the surgical fibrin sealant Vivostat compared with cisplatin solution (given at a concentration of 100 mg m\(^{-2}\)) in the same rat mesothelioma model that we used, Lardinois et al. reported platinum concentrations in the pleura ranging from 782.6 pg mg\(^{-1}\) to 1510.5 pg mg\(^{-1}\), lower than those that we detected in our animals [13].

The plasmatic platinum concentration in the animals treated with hyaluronate—chitosan cisplatin was also higher than that reported by the Swiss group (maximum at 6 days of about 1150 ng ml\(^{-1}\) compared with 14.9 pg \(\mu\)l\(^{-1}\), respectively). We think that this difference could be due to the pharmacokinetics of the polymeric films, which allows for a gradual cisplatin release and therefore a regular increase in the plasmatic levels of the drug.

Cisplatin concentration was so elevated locally (pleura) and systemically (serum) that, in the next experiment, we may consider reducing the dosage of the chemotherapeutic agent present in the film in order to decrease the general toxicity without losing the cytotoxic effect and anti-tumour response.

The loco-regional application of the polymeric film to patients with mesothelioma would be technically feasible and useful in different contexts. In the case of pleuropneumonectomy or pleurectomy/deortication, the film could be left in place to combat the residual microscopic disease. When the tumour has progressed and infiltrated the chest wall and mediastinal structures, rendering any surgical procedures worthless, the polymeric device could be intrapleurally applied as part of a multimodal therapy comprising systemic chemotherapy and hemithoracic radiotherapy. Considering the technical and pharmacokinetic properties of the polymeric films, they may also be suitable for peritoneal mesothelioma.

The next step will be to conclude the current study to confirm these preliminary results. Thereafter, we are interested in assessing the potential effect of pemetrexed loaded onto our polymeric films (alone and in association with cisplatin) in this rat mesothelioma model (protocol approved by the local veterinary committee). Recently, Greiller et al. performed a study on rats with the aim of evaluating the pharmacokinetics of pemetrexed administered intrapleurally compared with intravenous administration [25]. They concluded that intrapleural and intravenous routes provided similar area under the plasma concentration—time curve (AUC) and total body clearance but a significantly lower maximum plasma concentration, and therefore might improve patient tolerance to pemetrexed.

5. Conclusions

Hyaluronate—chitosan films loaded with cisplatin were significantly more effective in reducing tumour recurrence and assuring higher and prolonged plasmatic drug concentrations than cisplatin solution, without increasing systemic toxicity. Further studies are planned to confirm these encouraging data.

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References


Appendix A. Conference discussion

Dr W. Weder (Zurich, Switzerland): First of all, I want to declare, as you may probably have already realized, that Dr Ampollini worked as a research fellow in our laboratory, together with Isabelle Optiz, and was exposed to this model. He has to be congratulated that after his return to Parma that he built up his own research program there, and this is the first report from this, and this is exactly what we need, young thoracic surgeons who come along with their own research programs.

For the first time he presents a novel carrier for transporting chemotherapy. He used a polymer, as he has presented now, and he used cisplatin in order to do local control for malignant pleural mesothelioma. This concept could also apply for other tumours, such as lung cancer, where local recurrence rate should be high.

I have three questions for you. The first question is regarding the property of this new polymer which you have just presented. How is this material, which was developed for pleural mesothelioma, going to be used in other malignancies, such as in lung cancer? It is difficult to explain for me. And I was wondering in this context, if you used it to do local treatment or if it glue itself. Does it stick by itself or do you just stuff it into the cavity, and if this is the case, how would you manage this when you want to treat a big cavity such as in a human?

And then the second question:

(Appendix A continued)
Regarding the second question about the concentration of cisplatin in the serum; that is a very good point. First of all, the high cisplatin serum concentration that we could detect didn’t result in major systemic toxicity, and this is a very important issue to take into consideration. On the other side, there are some papers published in the literature reporting similar plasma concentration after intrapleural application of chemotherapeutic drug and intravenous administration of the drug. And regarding this issue, there is a very nice paper which I mentioned before by the group of Philippe Astoul in France. They performed a pharmacokinetic study in a rat model comparing the intrapleural and intravenous administration of pemexetred, and they found that the total systemic exposure to the drug was similar between intravenous and intrapleural routes. And that means that the area under the plasma concentration time curve and the total body clearance was similar after the two way of administrations. Therefore, this systemic delivery might allow the chemotherapeutic drug to diffuse into the inner core of the tumour through tumour neo-vascularisation, and, if so, the intrapleural administration of the chemotherapeutic drug might combine two modes of action: a direct exposure of the most superficial tumour cells and an indirect exposure of the deeper tumour cells to chemotherapy.

Coming to the third question about the cisplatin concentration in the tissue, that also is a very nice point. As I said before, these are preliminary results, so we are still working on that. I don’t have a definitive answer. But chitosan is a polymer that is insoluble in aqueous medium, and the release of the drug is essentially due to an enzymatic biodegradation of the film matrix. So theoretically it is possible to find some matrix compounds and matrix components in the pleural tissues that we harvested at the time point of animal’s autopsy. So, as Professor Weder hypothesized in his question, it could be a contamination of the carrier.

On the other hand, before starting the in vivo studies we performed extensive in vitro studies, and we found that after 6 days, the amount of drug released from the chitosan film was about 20 to 23% of the total. At the time point of autopsy, we removed the chitosan films that were still present in the pleural cavity, and our pharmacists found that about 70 to 73% of the drug was still present in the polymeric film. This means that the remaining part of the drug had not been released.

Dr I. Opitz (Zurich, Switzerland): I have a question. How do you load cisplatin to the carrier and how is it guaranteed that it is exactly 100 mg? Did you measure cisplatin concentration in this hyaluronate or chitosan carrier before you started the intrapleural application?

Dr Ampollini: We performed extensive in vitro studies and our pharmacists and pharmacologists produced the polymeric films of hyaluronate and chitosan, and the doses of cisplatin loaded to the film is exactly 100 mg/m² or 3 mg/kg. They determined it by HPLC.