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Original Paper

Pharmacokinetic Differences Between Rat Tumour and Lung Tissues Following Isolated Lung Perfusion with Cisplatin

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Isolated lung perfusion has been performed for the treatment of unresectable lung tumours; however, the pharmacokinetics of this procedure remain unclear. This study was conducted to investigate the changes in antitumour drug concentrations in tumour and lung tissues after isolated lung perfusion, using different perfusion times and perfusate drug concentrations. Isolated left lungs were perfused for 20, 40 or 60 min with 25, 50 or 100 μg/ml of cisplatin after solitary lung tumour nodules were established in rats, and the total platinum concentrations in the perfused lung and tumour tissues were determined by flameless atomic absorption spectroscopy. The oedema in the perfused lung tissues was evaluated by histological examination and by the wet to dry weight ratios of the lungs. The total platinum concentration increased significantly with perfusion time and increasing perfusate cisplatin concentrations in the lung tissue, but it did not change in the tumour tissue. The wet to dry weight ratios of the lung tissues did not differ significantly among the perfusion groups. Oedema of the perfused lung tissue did not change significantly with the perfusion time or perfusate cisplatin concentration. The results of this study indicate the possibility that different pharmacokinetics exist between tumour and lung tissues following isolated lung perfusion with cisplatin, which could be used as a clinical guide for the selection of appropriate perfusion times and perfusate drug concentrations.

Key words: pharmacokinetics, isolated lung perfusion, lung, tumour, cisplatin
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INTRODUCTION

ISOLATED LUNG perfusion was first developed successfully in a canine model in the 1960s [1, 2]. Theoretically, it is a more ideal and effective method of delivering regional chemotherapy to lung tumours than systemic chemotherapy because a higher concentration of antitumour agent can be directed to the tumour tissue; resulting in lower systemic toxicities. The safety and pharmacokinetic advantages of the isolated lung perfusion procedure have recently been demonstrated in experimental dog and rat models [3–6]. Moreover, satisfactory antitumour efficiency has also been achieved by isolated lung perfusion with doxorubicin or FUDR 2′-deoxy-5-fluorouridine in rat pulmonary micrometastatic sarcoma or colon cancer models [7–9]. Nevertheless, this method of regional chemotherapy is still not widely accepted for the clinical treatment of lung tumours. It is well known that the existence of micrometastatic tumour in the lung is difficult to diagnose; however, the majority of clinicians and patients would not select this invasive method of administering regional chemotherapy as treatment to prevent lung metastases. In fact, almost all of the clinical trials conducted on this method of delivering regional chemotherapy were performed on patients with inoperable primary or metastatic pulmonary tumours, but not micrometastatic lesions [10–12]. In these trials, different perfusion conditions and perfusate drug concentrations were given, which resulted in different outcomes [10, 11].

In a previous study, we observed efficacy using this regional chemotherapy method in a rat lung tumour node with visible growth was perfused with cisplatin [13]. Our results provided the possibility to extend this regional chemotherapy method to patients with unresectable primary or metastatic tumour. In this study, we performed isolated lung perfusion

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with cisplatin in a solitary rat lung tumour model to investigate the pharmacokinetic changes that occur with different perfusion times and perfusate drug concentrations in tumour and lung tissues.

MATERIALS AND METHODS

Animals

Fischer 344 male rats weighing 210–260 g were used in all of these experiments which were approved by the Institutional Animal Care and Use Committee of Yamaguchi University. Rats were bred in a standard laboratory and allowed free access to food and water in a temperature controlled environment with a 12-h light and dark cycle.

Establishment of the solitary lung tumour nodule

The methylcholanthrene(MCA)-induced rat sarcoma cell line was kindly supplied by Memorial Sloan-Kettering Cancer Center, New York, U.S.A. It is locally invasive in the lung, but rarely metastasises spontaneously [14]. Tumour cells were inoculated subcutaneously into rats and tumour was harvested when it had grown to 1.5–2.0 cm in diameter. A single tumour cell suspension was then prepared at a density of 5.0×10^7 cells/ml of viable tumour cells.

Solitary tumour nodules were established by a method previously described by Wang and associates [15]. Animals were anaesthetised with 50 mg/kg of pentobarbital intraperitoneally and trachea intubation was carried out with a 16F intravenous catheter [16]. The animals were ventilated by a volume ventilator (Harvard Rodent Ventilator, Model 683) with room air at a tidal volume of 10 ml/kg and a rate of 80 strokes/min. A left thoracotomy approximately 10 mm long was made through the seventh intercostal space, and the thoracic cavity was opened. A 27-gauge needle attached to a 100 µl microsyringe was inserted into the left lower lung at an angle of about 15° to a depth of 3 mm, and 1.0×10^6 tumour cells in 20 µl phosphate-buffered saline were inoculated into the lung parenchyma. After the lung was expanded, the chest and thoracotomy incision were closed.

Isolated left lung perfusion

Isolated left lung perfusion was performed by the method described by Weksler and colleagues [4]. Briefly, animals were anaesthetised and intubated, and ventilation was maintained as described above. A left thoracotomy was performed through the fourth intercostal space and the left pulmonary artery and vein were exposed under an operative microscope. A PE-10 catheter was inserted into the pulmonary artery for infusion, pulmonary venotomy was performed, and the effluent was collected by a suction catheter placed in the proximity of the venotomy.

Table 1. *Total platinum concentrations in tumour tissue after* 20, 40 *and* 60 *min ILP with* 25, 50 *or* 100 µg/ml *of cisplatin*

Perfusate cisplatin concentration	Total platinum concentration (µg/g tissue)		
	20 min ILP	40 min ILP	60 min ILP
25 µg/ml	4.17 ± 0.82	4.63 ± 0.69	4.76 ± 0.60
50 µg/ml	4.48 ± 0.83	4.37 ± 0.55	4.95 ± 0.80
100 µg/ml	4.66 ± 0.80	4.91 ± 1.02	4.84 ± 0.74

Data are expressed as means ± standard deviation. ILP, isolated lung perfusion.

Experimental design

The rats were randomised into nine groups 2 weeks after the tumour cells had been inoculated into the left lung. The left lung was perfused with cisplatin (Sigma, St. Louis, MO, U.S.A.), dissolved in 6% buffered hetastarch solution [17], at different concentrations of 25, 50 or 100 µg/ml, and for perfusion times of 20, 40 or 60 min. All groups were perfused at a same rate of 0.5 ml/min using a pump (Atom Infusion Pump 235, Tokyo, Japan), followed by 5 min washout with 6% buffered hetastarch solution [3, 18]. The animals were killed after the perfusion had finished and the tumour nodule in the left lung was excised. Samples of the tumour and the left lung tissue without tumour were collected and weighed. To determine the pharmacokinetic changes of isolated lung perfusion in tumour and lung tissue, only rats in which a lung tumour nodule had grown to 20–30 mg in weight were included in the final analysis. There were five to six rats in every group. The total platinum concentrations in the tumour and left lung tissues were determined by frameless atomic absorption spectroscopy.

To evaluate the oedematous changes of the left lung tissues, wet to dry weight ratios of the perfused lung tissues were determined for all the perfusion groups, and histological analysis was performed in lung tissues which had been exposed to the shortest time of 20 min perfusion with the lowest cisplatin dose of 25 µg/ml, or the longest time of 60 min perfusion with the highest cisplatin dose of 100 µg/ml.

Data analysis

Statistical analysis was performed by the paired *t*-test. All data are presented as means ± the standard deviation and significance was defined as $P<0.05$.

RESULTS

A solitary lung tumour nodule of approximately 4 mm in diameter had developed successfully in all the rats 2 weeks after the inoculation of 1×10^6 sarcoma cells into the left lower lung, and no pleural adhesion induced by the thoracotomy was found. None of the rats died during the perfusion period even when 60 min isolated lung perfusion was given. No significant difference in tumour nodule weight existed among the groups by statistical analysis.

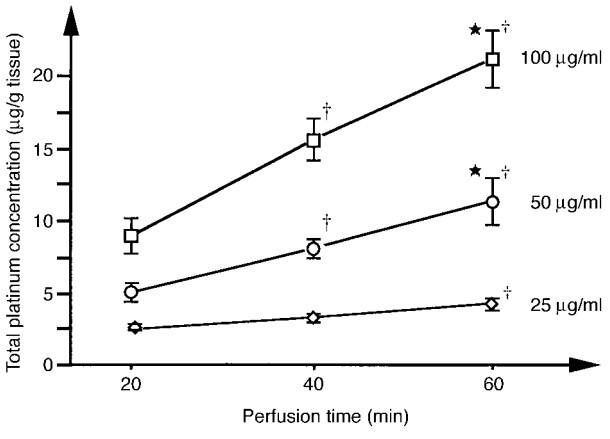

Figure 1. Total platinum concentration changes with perfusion time in the lung tissue. Data are expressed as means ± standard deviation (*$P<0.05$ versus 40 min perfusion, $P<0.05$ versus 20 min perfusion in each group with different cisplatin concentrations in the perfusate).

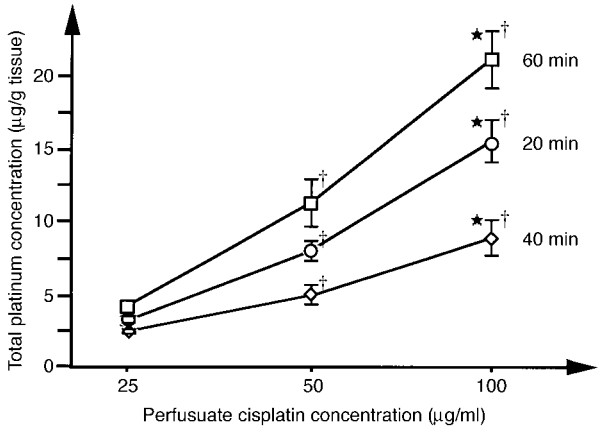

Figure 2. Total platinum concentration changes with perfusate cisplatin concentrations in the lung tissue. Data are expressed as means ± standard deviation (*$P<0.05$ versus 50 μg/ml cisplatin concentration, †$P<0.05$ versus 25 μg/ml cisplatin concentration in each perfusion group with different perfusion times).

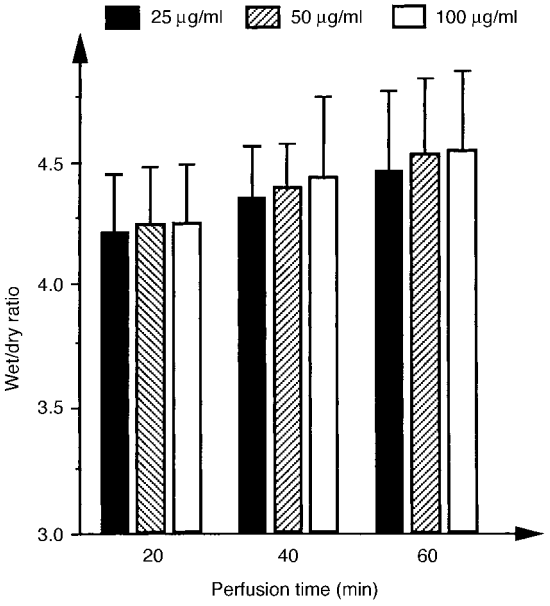

Figure 3. The wet to dry weight ratios of lung tissue after 20, 40 and 60 min perfusion with 25, 50 or 100 μg/ml of cisplatin. Data are expressed as means ± standard deviation.

Table 1 shows the total platinum concentrations in the tumour tissue after isolated lung perfusion with different perfusion times and different perfusate cisplatin concentrations. The total platinum concentrations in the perfused tumour tissues ranged from 4.17 ± 0.82 to 4.95 ± 0.80 μg/g tissue in all the perfusion groups, and did not change significantly according to either the perfusion time or perfusate cisplatin concentration. Conversely, the total platinum concentrations in the perfused left lung tissue increased significantly with the perfusion time (Figure 1) and perfusate cisplatin concentration (Figure 2).

No significant differences in the wet to dry ratios of perfused lung tissues were observed among the perfusion groups (Figure 3), although they increased slightly with the perfusion time. Histological analysis did not reveal evidence of severe oedema in the lung tissue, even after 60 min perfusion with 100 μg/ml of cisplatin, and no significant difference in histological lung damage was observed by light microscopy between the group given 20 min perfusion with 25 μg/ml cisplatin and the group given 60 min perfusion with 100 μg/ml cisplatin (Figure 4).

DISCUSSION

The lung is one of the most common sites of primary and metastatic malignant tumours. Although complete surgical resection is accepted as the most effective treatment, approximately 80% of patients with primary lung cancer [19] and the majority of patients with lung metastatic carcinoma [20] are not able to undergo complete surgical resection, as metastatic foci may exist in other organs, or multiple lesions are localised in the lung. Unfortunately, systemic chemotherapy and radiotherapy result in a very poor outcome due to dose-limiting systemic toxicities. In contrast, isolated lung perfusion facilitates the administration of high doses of antitumour agent to the perfused tissue whilst limiting the systemic toxicities [3]. Furthermore, we have previously demonstrated from our studies in rats the possibility that this method of regional chemotherapy may be suitable for patients with an unresectable primary or metastatic tumour in the lung [13]. However, when given with doxorubicin, cisplatin or TNF-α for the treatment of diffuse bronchioloalveolar carcinoma or lung metastatic sarcoma [10–12], the antitumour potency expected in some clinical practices was not achieved. These clinical trials indicated that it was necessary to improve the regional chemotherapy method of isolated lung perfusion.

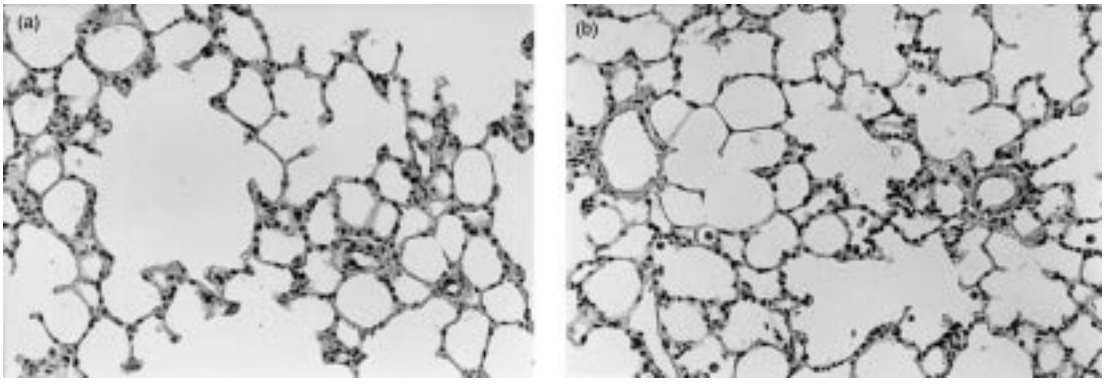

Figure 4. Histological evaluation of left lung tissue; (a) after 20 min isolated long perfusion (ILP) with 25 μg/ml of cisplatin; and (b) after 60 min perfusion with 100 μg/ml of cisplatin. No significant difference in lung oedema or other evidence of lung damage was found in either of these perfusion groups (H & E staining, original magnification ×150).

We observed in a rat lung tumour model that the total platinum concentration in tumour tissue was significantly inversely related to the weight of tumour nodules after isolated lung perfusion with cisplatin (data not shown). This result indicated the possibility that isolated lung perfusion treatment was more effective against small tumours than large ones because the antitumour potency is dose-dependent for the majority of antitumour drugs. Moreover, we previously advised that large lung tumour lesions should be debulked prior to isolated lung perfusion in patients with an unresectable lung tumour, in accordance with Ratto and associates [11].

To investigate the pharmocokinetic changes of isolated lung perfusion with different perfusion times and perfusate drug concentrations in perfused tumour and lung tissues, we selected 20, 40, and 60 min as the perfusion times because the longest perfusion time tolerated by rats is approximately 60 min, whilst concentrations of 20, 50, and 100 μg/ml of cisplatin were chosen as the perfusate because cisplatin is known to be an effective antitumour agent against both primary and metastatic pulmonary carcinomas [21, 22] and the highest concentration tolerated by rats in isolated lung perfusion treatment is 100 μg/ml [13]. We observed that the total platinum concentration in perfused lung tissues increased significantly with the perfusion time and perfusate cisplatin concentration. This was similar to the results observed following isolated lung perfusion with doxorubicin or FUDR in normal rat lungs [3, 9, 17]. However, no significant difference was found in the wet to dry weight ratios of perfused lung tissues among the perfusion groups in this study, although a slight increase with perfusion time was observed. Moreover, histological analysis did not reveal any significant lung oedema in lung tissues subjected to the longest time of 60 min perfusion with the highest dose of cisplatin of 100 μg/ml, even though it was possible that severe lung damage could have occurred in the reperfusion period. It did not seem likely that the increase of total platinum concentrations in the perfused lung tissues resulted from oedematous changes of the perfused lung tissues. Conversely, the total platinum concentration in tumour tissues did not change with perfusion time or perfusate cisplatin concentration when tumour nodules of similar size were examined for statistical analysis. The different changes in total platinum concentrations between tumour and lung tissues indicated that various pharmacokinetic differences existed following isolated lung perfusion regional chemotherapy.

One of the possible factors inducing the different pharmacokinetic changes was the different blood supplies from the pulmonary or bronchial circulations to the lung and tumour tissues. Isolated lung perfusion is a method of giving regional chemotherapy by infusing an antitumour drug via the pulmonary artery; therefore, the drug concentration in the perfused tumour and lung tissues is determined at least partially by the pattern of blood supply to these tissues. According to investigations conducted by Milne in humans and rats, the source of blood in pulmonary metastatic deposits may be supplied by pulmonary circulation, bronchial circulation or a combination of both [23–25]. Indeed, tumour nodules were stained after trypan blue infusion both from the pulmonary artery or aorta; although the consistency of the staining in separate tumour nodules was not different, suggesting that the blood supply was derived from both the pulmonary and bronchial circulations in the tumour model (data not shown).

Cisplatin and its platinum-containing products are rapidly and extensively bound to tissue and plasma proteins, including albumin, γ-globulins and transferrin. As binding to tissue and plasma proteins appears to be essentially irreversible, and protein binding increases with time [26], this suggests that the different pharmacokinetic changes between the perfused tumour and lung tissues in this study were possibly induced by differences in protein expression in the tumour and lung tissues. In other words, different platinum-binding proteins were contained in tumour and normal lung tissues, and (in the tumour) platinum-binding proteins were saturated after 20 min perfusion with 25 μg/ml of cisplatin, but were not saturated in the lung tissue even after 60 min perfusion with 100 μg/ml of cisplatin.

Our investigation showed that the antitumour drug concentration in the tumour tissues did not increase with either perfusion time or perfusate cisplatin concentration. This suggests that the antitumour potency would not increase with an extension of the perfusion time and/or an increase of perfusate drug concentration when cisplatin was used to perfuse a tumour mass. According to our results, further studies are required to raise the drug concentration to the tumour tissue and relieve the toxicity to perfused lung tissue by improving the perfusion conditions when isolated lung perfusion regional chemotherapy was used in patients with unresectable primary or metastatic pulmonary carcinoma.

In summary, we observed different pharmacokinetics in tumour and lung tissues following isolated lung perfusion with cisplatin in a lung sarcoma tumour rat model, although this observation has not been confirmed in other tumour models perfused with different drugs, or in clinical trials. Our findings provide a guide for selecting appropriate perfusion times and perfusate drug concentrations in clinical trials because similar pharmacokinetic changes could occur in isolated lung perfusion regional chemotherapy in patients with unresectable primary or metastatic lung tumour.

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