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Pharmacokinetics and the effect of heat on intraperitoneal pemetrexed using a murine model

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ABSTRACT

Background: Pemetrexed is a systemic chemotherapeutic agent used in the treatment of malignant mesothelioma. This drug represents a potentially promising intraperitoneal (IP) agent to use for hyperthermic intraperitoneal chemotherapy (HIPEC) in the treatment of peritoneal mesothelioma. However, this has yet to be supported by preclinical studies. Therefore, we aimed to study the effect of pemetrexed dose and perfusion temperature on the resultant pemetrexed concentration in 3 different compartments (systemic circulation, portal circulation and peritoneal tissues) using a murine model.

Methods: Under general anesthesia, 29 Sprague-Dawley rats were submitted to 3 different doses of IP pemetrexed (500, 1000 and 1500 mg/m^2) combined with 3 different perfusion temperatures (37, 40 and 43 °C) for a total duration of 25 min. At the end of perfusion, samples in different compartments (systemic circulation, portal circulation and peritoneum) were harvested and concentrations of pemetrexed were measured using high performance liquid chromatography.

Results: With increasing dose of IP pemetrexed, higher concentrations were measured in the 3 compartments tested. In peritoneal cells, the difference between IP doses of 500 and 1000 mg/m^2 (2.03 vs. 19.17 μg/g, $p < 0.001$) was greater than the difference between 1000 and 1500 mg/m^2 (19.17 vs. 22.80 μg/g, $p = 0.027$). When the perfusion temperature increased, we observed a proportional rise of pemetrexed concentration in both the portal and systemic compartments; while in the peritoneal cells, the pemetrexed concentration increased up to 40 °C, after which it plateaued.

Conclusion: Both heat and increasing doses of IP pemetrexed enhance peritoneal cell concentration of pemetrexed. However, for temperatures above 40 °C, pemetrexed concentration reached a plateau in peritoneal cells. Systemic and portal concentrations increased proportionally with both increasing temperatures and IP doses. We believe these results should be taken into consideration for the design of an eventual clinical study in humans.

1. Introduction

Malignant mesothelioma is a rare type of cancer that arises most commonly from the pleura, but can also develop from the peritoneum, the pericardium and the tunica vaginalis of the testis. Its mortality is associated with local spread as distant metastases are uncommon. Median survival of patients with untreated mesothelioma is estimated to be between six to twelve months [1].

Palliative surgery and systemic chemotherapy with or without radiotherapy were traditionally used to treat malignant peritoneal mesothelioma (MPM) and gave patients an additional nine to fifteen months of survival [2–4]. With the advent of cytoreductive surgery (CRS) combined to hyperthermic intraperitoneal chemotherapy (HIPEC), survival of patients with MPM has greatly improved, now averaging from 29.5 to 92 months [4–6]. The intraperitoneal (IP) drugs most widely used in this setting are cisplatin, doxorubicin or mitomycin-C. However, these molecules have not been proven to have significant benefit in the treatment of mesothelioma as their efficacy was demonstrated in the treatment of adnexal and colorectal carcinomatosis.

Pemetrexed (Alimta®) is an antifolate metabolite that was FDA approved in 2004 for the treatment of pleural mesothelioma [7]. It was shown that when used in combination with cisplatin, it

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significantly increased survival of patients with MPM (to 24 months) [4,8]. Cytoreductive surgery combined to HIPEC with the addition of pemetrexed thus exhibits an interesting profile for the treatment of MPM. In 2000, Pestieau et al. compared the pharmacokinetics of intraperitoneal (IP) pemetrexed administration to the intravenous (IV) route using a murine model. They demonstrated that IP administration generated a 24-fold increase in the IP concentration of pemetrexed when compared with the IV route [9]. Then, in 2012, a phase II study showed that IP pemetrexed combined to IV cisplatin could be used as adjuvant treatment of MPM with low morbidity [10]. However, the dose and the perfusion temperature chosen in this study were not based on any pharmacokinetic evidence as the properties of pemetrexed in this setting are not well established, and the most efficient way to administer perfused IP pemetrexed is yet to be defined.

The objectives of the present study were to evaluate the effect of pemetrexed dose and perfusion temperature on the resultant pemetrexed concentration in 3 different compartments (systemic circulation, portal circulation and peritoneal tissues) using a murine model.

2. Material and methods

2.1. Study design

Twenty-nine male Sprague-Dawley rats weighing on average 265 g (range: 228–383 g) with a mean body surface of 0.036 m^2 (range: 0.033–0.046 m^2) were used. Each rat was submitted to one dose of IP pemetrexed at set temperature for 25 min. As shown in Table 1, five groups of rats were submitted to different experimental conditions.

Doses of IP pemetrexed were 500, 1000 and 1500 mg/m^2. Based on existing FDA recommendations, we selected our initial dosage of pemetrexed at 500 mg/m^2, being the maximum tolerated systemic dose in humans. We then selected dosages that were twice and three times the initial dosage recommended to enhance pemetrexed detection in different body compartment (systemic circulation, portal circulation and peritoneal tissues).

We used 3 different perfusion temperatures (37, 40, and 43 °C). We chose not to exceed 43 °C as we previously demonstrated that perfusion temperatures exceeding 43 °C were associated with mortality rates above 50% [11].

Our Animal Protection Committee approved this study before performing the experiments.

2.2. Surgical procedure and tissue harvesting

All rats were put under general anesthesia using isoflurane inhalation in a standardized fashion. Two multi-perforated catheters (17 French Levine tubes with extremities connected to standard intravenous lines with injection ports) were then placed in the abdominal cavity via two 5 mm incisions and fixed with watertight purse-string sutures.

To monitor inflow and outflow temperatures, two thermistors probes were fixed inside the catheters and connected to a digital thermometer. Prior to insertion, all circuits were filled with normal saline solution in order to eliminate air from the circuit. One catheter tip was placed in the upper abdomen (inflow) and the other in the lower abdomen (outflow) to maximize peritoneal exposure to the perfusate. Tips of catheters were coated with gauze to prevent occlusion by intra abdominal contents. To prevent heat loss, rats were placed on heated mattresses that generated heat in accordance to the rectal temperature (Fig. 1).

The abdominal cavity was then filled with 60 cc of isothermic normal saline solution and closed circuit fluid circulation was then initiated with a peristaltic pump. The perfusate was heated by having a standardized length of tubing resting in a thermostatic bath. Pump flow was adjusted to enable the total volume of the circuit (tubing 40 cc + peritoneal cavity 60 cc) to circulate 3 times during the 25-min perfusion. Once the desired intra abdominal temperature was obtained, pemetrexed was administered through an injection port near the inflow catheter. From this point, the perfusion was maintained for 25 min (t25) under continuous temperature surveillance, which was displayed with one tenth of °C precision on a digital thermometer. Rhythmic massage of the abdomen was applied to favor a uniform distribution of heat and perfusate. At t25, the peristaltic pump was stopped and the intra-abdominal fluid was evacuated through the injection port. A midline laparotomy was performed and the peritoneal surfaces were rinsed with 60 cc of a normal saline solution. Two ml of blood were harvested from both the portal vein (portal blood) and inferior vena cava (systemic blood). These samples were centrifuged for 5 min allowing separation of the plasma and cellular elements which were then frozen separately at −80 °C. We then harvested

Table 1
Experimental groups according to doses and perfusion temperatures. n: number of subjects.

Temperature	Dose of pemetrexed		
	500 mg/m^2	1000 mg/m^2	1500 mg/m^2
37 °C		Group 1 (n = 6)	
40 °C	Group 4 (n = 6)	Group 2 (n = 5)	Group 5 (n = 6)
43 °C		Group 3 (n = 6)	

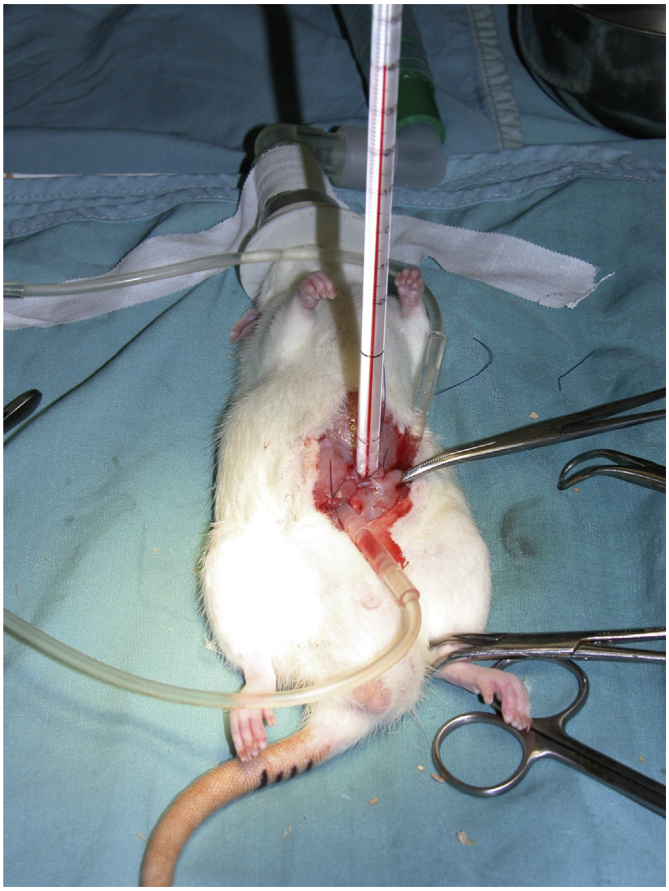

Fig. 1. Intraperitoneal pemetrexed infusion on a murine model.