Journal of Visceral Surgery (2017) xxx, xxx—xxx

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

ORIGINAL ARTICLE

A new animal model for hyperthermic intraperitoneal chemotherapy (HIPEC) in tumor-bearing mice in the treatment of peritoneal carcinomatosis of ovarian origin

G. Miailhe[a,c,1], A. Arfi[a,c,1], M. Mirshahi[c], C. Eveno[b,c], M. Pocard[b,c], C. Touboul[a,c,*]

[a] *Service de gynécologie obstétrique, hôpital intercommunal de Créteil, université Paris Est, Paris XII, 40, avenue de Verdun, 94000 Créteil, France*
[b] *Service de chirurgie digestive et cancérologique, hôpital Lariboisière Fernand-Widal, université Paris VII, 49, boulevard de la chapelle, 75010 Paris, France*
[c] *UMR Inserm U965, angiogenèse et recherche translationnelle, hôpital Lariboisière, 49, boulevard de la chapelle, 75010 Paris, France*

KEYWORDS
Hyperthermic intraperitoneal chemotherapy;
Peritoneal carcinomatosis;
Ovarian cancer;
Murine model;
Oxaliplatin

Summary
Aim of the study: We set out to develop and evaluate the morbidity of a non-invasive hyperthermic intraperitoneal chemotherapy (HIPEC) procedure in mice. HIPEC has been shown to improve overall survival in treating ovarian cancer with peritoneal carcinomatosis. However, related complications, toxicity and the lack of randomized trials limits its widespread use. To improve the surgical technique, there is a need for animal models that allow teams to work on large groups without burdensome logistics.
Materials and methods: To develop the model, we first determined optimal HIPEC conditions in 20 Black Six mice without carcinomatosis. To evaluate HIPEC morbidity, peritoneal carcinomatosis cells of ovarian origin were injected into the peritoneum of 10 pathogen-free Nude mice. The mice underwent HIPEC 21 days later under general anesthesia. An inflow catheter was introduced into the left hypochondria and an outflow catheter was introduced into the left iliac fossa. Bath infusion was oxaliplatin (920 mg/m^2) at 43 °C for 12 minutes. The mice were monitored and sacrificed two weeks after the procedure.
Results: No deaths were observed during the procedure and infusion was well tolerated throughout the HIPEC. One mouse died the day after the procedure. No major dehydration, hemoperitoneum or evisceration was observed.
Conclusion: This mouse model of closed abdomen HIPEC has limited morbidity and could be a useful model to study HIPEC regimens and its effects on peritoneal carcinomatosis.

* Corresponding author.
E-mail address: cyril.touboul@gmail.com (C. Touboul).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jviscsurg.2017.10.008
1878-7886/

Introduction

Epithelial ovarian cancer (EOC) is the leading cause of death from gynecological cancer in northwestern Europe and in northern America [1]. Most women are diagnosed only after peritoneal dissemination has occurred. EOC tends to be chemosensitive and confine itself to the surface of the peritoneal cavity for much of its natural history. Though these features make it an obvious target for intraperitoneal chemotherapy, standard treatment is complete cytoreductive surgery followed by five to eight cycles of intravenous taxane chemotherapy [2]. Even though initially effective, drug-resistant cancer or relapse from residual disease reduces the 5-year survival rate to about 20% [3].

Cisplatin is a DNA damaging chemotherapeutic used to treat solid tumors including EOC. However, resistance to cisplatin in this setting limits clinical success. Three large randomized clinical trials have shown the benefit of hyperthermia when administering chemotherapy agents intraperitoneally to target small remnant tumor load and putative malignant cells in the abdominal cavity, a technique known as hyperthermic intraperitoneal chemotherapy (HIPEC) [4—6]. Hyperthermia is tumoricidal alone [7] and has been shown to enhance cisplatin inhibition of peritoneal tumor growth [8]. A recent Cochrane database [9] has shown that HIPEC increases overall survival and progression-free survival in women with advanced ovarian cancer. However, the potential for catheter-related complications and toxicity needs to be taken into account. Efforts to define the role of HIPEC in a large randomized trial failed to attain the required number of patients because of patient dissatisfaction with randomization [10]. Moreover, optimal treatment modalities remain to be defined and targeted therapies developed in order to increase efficacy and decrease toxicity. Animal models are therefore needed.

While animal models have already been developed in rats and pigs to study HIPEC procedures [11], these are burdensome in terms of logistics and limit the possibility of working on large groups. Mice models are more accessible but published data are scarce and existing reports fail to include animal survival or postoperative complications because the mice were sacrificed immediately after the HIPEC procedure [12—14].

The objective of our study was to develop a new non-invasive HIPEC procedure using mice as a miniaturized animal model, and to evaluate the morbidity of the procedure.

Materials and methods

Animals

Twenty female C57BL/6 mice between 6—8 weeks old (Charles River, Arbesle, France) were used to build the experimental procedure and determine optimal conditions.

Ten female pathogen-free Nude mice (without thymus, T-lymphocyte free), weighing 20 to 27 g (average: 25.3 g) and between 7—9 weeks old (Charles River, Arbesle, France) were used to perform the HIPEC procedure on a peritoneal carcinomatosis model.

The animals were housed in filter-topped cages (5 mice per cage) under clean, non-sterile, standardized conditions (temperature 20—24 °C, relative humidity 50—60 percent, 12 h light/12 h dark cycle). They were fed with standard laboratory diet and tap water ad libitum.

Maintenance and care of all experimental animals were carried out according to the guidelines of the local Animal Protection Commission and in compliance with national guidelines (Saisine n°02095, Animal Protection Committee, French Ministry of Agriculture).

Cells and cell culture

We used the human epithelial ovarian cancer cell (OCC) line OVCAR-3. OCCs were transfected with a plasmide expressing luciferase. The OCCs were cultivated in an incubator at 37 °C and 5% CO_2 in monolayer cultures in 20 mL complete medium of Dulbecco's Modified Eagle's Medium (DMEM), 10% heat-inactivated fetal bovine serum, 1% Penicillin/Streptomycin, and 1% L-glutamate (Gibco, Saint-Aubin, France).

Establishment of intraperitoneal metastatic ovarian tumors in mice

The OVCAR-3 cell suspension (6.0×10^6 cells in 500 μL of serum-free DMEM media) was injected into the peritoneum in the left iliac fossa of mice using an 18-gauge needle.

Evaluation of carcinomatosis

To assess the peritoneal carcinomatosis before surgery, the mice were monitored twice a week by bioluminescence after intraperitoneal injection of 0.2 mL of Luciferine (150 ug/mL), 10 minutes before acquisition. Almost 3 weeks after injection (by day 19), the mice had developed disseminated intraperitoneal tumors.

Chemicals

Oxaliplatine (Onco-Tain™, Hospira), was chosen as chemotherapy for its peritoneous absorption qualities. The administered dose was 920 mg/m^2 according to the results of Piché et al. [15].

Experimental design

To develop the model, we first tested the procedure HIPEC in the 20 Black Six mice without carcinomatosis and determined optimal conditions. The following parameters were tested without chemotherapy in the bath liquid:

- safety and operative morbidity of open or closed abdomen intraperitoneal infusion;
- thermic control using a heat mattress: optimal temperature of the mattress and length of the procedure.

Before surgery, the mice were administered a subcutaneous injection of 0.5 mL buprenophine. They were then anesthetized with 4% isoflurane (2L/mn) in an inhalation chamber (Fig. 1A). The body temperature was monitored using a rectal temperature probe (Fig. 1B). The use of a warmed mattress to limit body heat loss was evaluated in four mice:

- mouse No. 1: no warmed mattress;
- mouse No. 2: warmed mattress at 35°C;
- mouse No. 3: HIPEC procedure without warmed mattress;
- mouse No. 4: HIPEC procedure with warmed mattress at 35 °C:
  - Inflow catheter: thermic control and optimal flow;
  - Outflow catheter: optimal flow.

In the second study, we tested HIPEC using oxaliplatin on mice with ovarian peritoneal carcinomatosis to evaluate the

Please cite this article in press as: Miailhe G, et al. A new animal model for hyperthermic intraperitoneal chemotherapy (HIPEC) in tumor-bearing mice in the treatment of peritoneal carcinomatosis of ovarian origin. Journal of Visceral Surgery (2017), https://doi.org/10.1016/j.jviscsurg.2017.10.008