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Original article

A study of peritoneal metastatic xenograft model of colorectal cancer in the treatment of hyperthermic intraperitoneal chemotherapy with Raltitrexed

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ABSTRACT

Peritoneal metastasis of colorectal cancer is one of the most incident and fateful diseases among relapse cases. It shows a certain resistance to systemic chemotherapy. The perfusion system in clinic is complex and hard to be used in fundamental researches. This study aims at evaluating the effect of an improved hyperthermic intraperitoneal chemotherapy with Raltitrexed used in tumor-bearing mice with peritoneal metastatic colorectal carcinoma. The results showed that no severe adverse effect was observed. All control animals developed extensive peritoneal and mesenteric metastatic nodes. Tumor sites in the treatment groups were reduced significantly. The administration dose of Raltitrexed influenced concentration in systemic blood and peritoneal tissues. Temperature promoted the intracellular absorption of Raltitrexed significantly. Our findings reveal that hyperthermic intraperitoneal chemotherapy is an efficient therapy in treating peritoneal metastatic carcinoma in nude mice. It can effectively reduce the extension of carcinoma cells from macro and micro examination. The combination of hyperthermia and Raltitrexed resulted in an improved therapeutic effect on animal models.

1. Introduction

Tumor metastasis has been well characterized as a primary tissue leading to poor prognosis in patients with cancers. Specific for advanced cancers in abdominal viscera, peritoneal metastasis is one of the main extension routes [1].

It has been found that the survival time of patients affected with gastrointestinal cancers is strongly associated with the peritoneal metastasis degree as well as the condition of the primary lesion [2,3]. Ten-thirty percent of operable patients will suffer from different degrees of invasion in the peritoneum after initial surgery, while the peritoneal metastasis rate for inoperable patients is even higher [4,5]. Although efforts have been put in elongating the survival time of patients with peritoneal metastasis, the long-term survival rate still remains poor [6]. This is largely due to the existence of peritoneum-plasma barrier, which limited the transmission of anti-tumor drugs from the blood vessel to peritoneum, and thereby restricted the efficacy.

It has been reported that the development of lymph is closely related to peritoneal metastasis [7,8]. In a rat model with mesenteric lymphatic vessel obstruction, a contact between lymph and vein was observed, and reflux intestinal lymph appeared in mesenteric lymph vessels, revealing that the carcinoma cells accumulated in lymph vessels would lead to peritoneal metastasis [9,10]. As a result, both mesenteric metastatic nodules and peritoneal metastatic nodules can serve as the main indicators of colorectal carcinoma peritoneal metastasis. This mechanism appeals intensive attention in the area of metastatic carcinoma.

In systemic chemotherapy, penetration effect of anti-tumor drugs on abdominal cavity is limited. By contrast, intraperitoneal chemotherapy has a distinct therapeutic effect on peritoneal metastatic nodules because the anti-tumor drugs are transmitted to the cavity directly. Thereby, tumor nodes are exposed to high concentration of drugs [11].

Hyperthermic intraperitoneal chemotherapy (HIPEC) is a treatment that fills the abdominal cavity with chemotherapeutic

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agents under accurate temperature and perfusion speed. HIPEC combines chemotherapy with thermotherapy in abdominal cavity to reach the destination of clearing away free cancer cells, subclinical lesions and tiny cancer nodules. Consequently, it has been playing an important role in the treatment of peritoneal metastatic colorectal carcinoma in clinical practice [12]. The synergistic effects of hyperthermic that straightly targets cancer cells, anti-cancer drugs and mechanical flushing action play the main roles in the treatment [13,14].

During the hyperthermic intraperitoneal chemotherapy, the cytotoxic drug in hyperthermic status imposed restrictions on depth of penetration [15]. Therefore, drugs are gathered in abdominal cavity. Metastasis nodes are limited to minimum.

Thymidylate synthetase plays an important role during the synthetic process of thymidine triphosphate (TTP) to form DNA. As a result, the thymidylate synthetase inhibitor, Raltitrexed (RTX), can produce DNA fragment, and then causes the cells to death [16]. Pharmacological studies suggest that compared with fluorouracil, RTX is more favorable due to its better clinical safety [17]. Therefore, RTX is frequently applied to treat with advanced colorectal cancer cases with peritoneal metastasis to improve the safety [18–21]. In clinical practice, RTX is generally administrated through intravenous drip. Recent years, clinical trials of RTX hyperthermic intraperitoneal perfusion chemotherapy are performed to explore the efficacy [22].

In this study, considering the defects of animal models for the investigation of HIPEC at present, we employed an improved perfusion system on tumor-bearing nude mice to investigate whether the tumor-suppressive efficacy of RTX could be increased by HIPEC. Perfusion through puncture needles was used to replace with laparotomy to reduce the risk of infection. At the same time, RTX was selected as the studied agent. Our study explored the impacts of temperature and administration method on the efficacy of RTX during the perfusion. Further analyses showed that our perfusion system was of good tolerance and promoted the drug absorption. Our study provides an easy and practical perfusion model on nude mice for further preclinical studies, and confirms the conclusion that RTX can be well used in HIPEC system.

2. Methods

2.1. Animals

Nude mice with half males and half females were purchased from Shanghai Slac Laboratory Animal Center. The animals were kept in a sterile environment during the experiment with 12 h light every day. All the animals were maintained and taken care of according to the Institutional Animal Care Guidelines.

2.2. Cell lines and cell culture

Human colorectal carcinoma cell lines HCT-116 and LOVO were purchased from the Cell Bank of Chinese Academy of Science and cultured in RPMI 1640 medium (Gibco industries) with 10% (vol/vol) heat inactivated fetal bovine serum (Gibco industries), penicillin (100 units/mL) and streptomycin (100 μg/mL) under 37 °C, 5% CO_2 atmosphere.

2.3. Cell viability assay

Cell viability was measured by MTT assay. For experiments, cell lines HCT116 and LOVO were seeded onto 96-well cell culture plates and incubated for 24 h. RTX was then added to a concentration of 0 μM (control), 6.25, 12.5, 25 or 50 μM. Hyperthermia was applied on cells at 43 °C for 0, 0.5, 1, 2 or 4 h in an atmosphere of 5% CO_2 respectively. Incubation was continued for 48 h at 37 °C in the incubator. Then, MTT solution was added to cells in 96-well plates. The optical density was read at 450 nm. IC50 values were calculated by software Graph Pad Prism.

2.4. tumor xenograft

For the xenograft model, luciferase-expressing human colorectal carcinoma cell line 116-luc was re-suspended in PBS containing 500 μg/mL Matrigel (BD Matrigel Basement Membrance Matrix), and the final concentration was 2.5×10^7 mL^{-1}. For each mouse, 100 μL cell suspensions were injected into the middle of abdominal cavity.

2.5. Chemotherapeutic regent

RTX was supported by Chia Tai Tianqing Pharmaceutical. For *in vitro* assays, the drug was dissolved in sterile water at the concentration of 1 mg/mL, and stored in dark under −20 °C. For *in vivo* assays, the drug was dissolved into appropriate concentrations using physiological saline, and then preserved under the same condition above.

2.6. Hyperthermic intraperitoneal chemotherapy

In the first experiment, 36 animals were randomly assigned into 6 groups. The specific administration situations were shown in Table 1.

HIPEC system consisted of a heat exchanger device and two electronic peristaltic pumps. The heated perfusate was driven by two roller pump synchronously to flow in and out of the system. At the end, the perfusion tubes were connected with puncture needles. Before perfusion, the mice were fasted overnight. The animals were anaesthetized with 1% pentobarbital sodium, and fixed at operation table on supine position. The abdominal wall skin was sterilized with 75% alcohol. Two puncture needles were fixed on the left and right of the abdomen apart, aiming at simulating the clinic procedure of perfusion.

To monitor the temperature during the process, two thermocouples were fixed at both of the perfusion sites. The hyperthermic intraperitoneal perfusion lasted for 30 min after the intended

Table 1
Distribution of raltitrexed in mice is according to temperature, administration route and dose in the experiment.

	Temperature °C	Administration route	Dose mg m^{-2}
Group I	37	Peritoneal perfusion with physiological saline	0
Group II	37	Tail vein with RTX	2
Group III	37	Peritoneal perfusion with RTX	3.75
Group IV	37	Peritoneal perfusion with RTX	7.5
Group V	43	Peritoneal perfusion with RTX	3.75
Group VI	43	Peritoneal perfusion with RTX	7.5