RESEARCH ARTICLE

Experimental Study on Sustained-release 5-Fluorouracil Implantation in Canine Peritoneum and Para-aortic Abdominalis

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Abstract

Objective: To observe local and systemic toxicity after sustained-release 5-fluorouracil (5-Fu) implantation in canine peritoneum and para-aortic abdominalis and the changes of drug concentration in the local implanted tissue with time. Methods: 300 mg sustained-release 5-Fu was implanted into canine peritoneum and para-aorta abdominalis. Samples were taken 3, 5, 7 and 10 days after implantation for assessment of changes and systemic reactions. High performance liquid chromatography was applied to detect the drug concentrations of peritoneal tissue at different distances from the implanted site, lymphatic tissue of para-aortic abdominalis, peripheral blood and portal venous blood. Results: 10 days after implantation, the drug concentrations in the peritoneum, lymphatic tissue and portal vein remained relatively high within 5 cm of the implanted site. There appeared inflammatory reaction in the local implanted tissue, but no visible pathological changes such as cell degeneration and necrosis, and systemic reaction like anorexia, nausea, vomiting and fever. Conclusions: Sustained-release 5-Fu implantation in canine peritoneum and para-aortic abdominalis can maintain a relatively high tumour-inhibiting concentration for a longer time in the local implanted area and portal vein, and has mild local and systemic reactions. Besides, it is safe and effective to prevent or treat recurrence of gastrointestinal tumours and liver metastasis.

Keywords: Sustained-release 5-Fu - interstitial chemotherapy - peritoneum - para-aortic abdominalis - implants

Asian Pac J Cancer Prev, **15 (1)**, 407-411

Introduction

The incidence of gastric cancer tops the list among malignant gastrointestinal tumours, and its mortality is also very high (Wang et al., 2013; Wei et al., 2013). The average mortality of gastric cancer in China has reached to 20 per 100, 000 people. However, the intra-abdominal recurrence and liver metastasis are the leading causes of deaths in patients with advanced gastrointestinal tumours and the difficult problems and research hotspots in the treatment of malignant gastrointestinal tumours at present (Zhou et al., 2008; Zhang et al., 2011). Systemic chemotherapy and intraperitoneal hyperthermic chemotherapy are the current treatment for malignant gastrointestinal tumours, but the patients eventually abandoned the treatment because of sever toxicities or unideal efficiency (Muchmore et al., 1996). The intraperitoneal recurrence is mainly due to exfoliated cells in the peritoneal cavity, retroperitoneal lymph nodes and metastatic lesions in the peritoneum. The ideal chemotherapy to treat gastrointestinal tumours should be conduct effective treatment in commonly-encountered recurrent and metastatic parts such as resected parts, lymph node metastasis, peritoneal implantation and liver. Hence, the key to successfully treat gastrointestinal tumours is to find ways to increase the concentration of chemotherapy drugs and prolong its action duration in the lymph nodes, peritoneum and portal vein. 5-fluorouracil (5-Fu) is the most common and effective chemotheray drug used to treat gastrointestinal tumours. Characterized by long action duration, reaching the maximum drug concentration in targeted lesions, reducing the systemic toxicity and absorption not through gastrointestinal tract, so sustained-release 5-Fu can creduce the drug loss and maximize drug utilization. In this experiment, sustained-release 5-Fu was implantated in canine peritoneum and para-aortic abdominalis, the results indicated that 10 days at least after implantation, a relatively high drug concentration in the local implanted area and portal vein was maintained, and there were no conspicuous reactions in the local part, all of which provide experimental evidences for advanced gastrointestinal tumours.

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Materials and Methods

Preparation of the implanted sustained-release 5-Fu

The preparation process of the implanted sustained-release 5-Fu (Chinese Wuhu Zhongren Pharmaceutical Co., Ltd., Batch Number: 20030615) was as follows: First, put 5-Fu particles (the diameter of particles within ϕ0.1~0.5 mm) into the coating pan with specially-made coating pan after crushing and sieving (120 meshes) the active ingredients in 5-Fu on the clean workbench; next, spray medically-used polydimethylsiloxane solution into the coating pan in a few times to form the microcapsules with 5-Fu microsphere as the core and silicone rubber as the envelope, and then mix with polydimethylsiloxane solution again after drying and curing 4 h at 50°C, press into the mold (ϕ0.8×4 mm) and put it on the side to solidify for 24 h; at last, dry for 4 h under 40°C and 0.09 MPa, then package after inspection and sterilization.

Determination of releasing rate: First, soak in the normal saline at 37°C for 15 days, and the drug release >85%; next, implant the drug rods (the shape ϕ0.8×4 mm) with 2 mg active ingredients into the sacral spinal muscle of 8 rats, and then kill them after 15 days; finally, take out the drug rods and analyse the drug residue to calculate drug release being 86%~91%.

Animal preparation

16 healthy experimental canines (provided by Experimental Animal Centre, Anhui Medical University) with body weight 10~12 (10.3±1.47) kg were randomly divided into 4 groups, in which females and males were 6 and 10, respectively. Before the experiment, animals were allowed to adapt the experimental environment for 12~14 h, with preoperative fasting and room temperature being 20~25°C. (This experiment was licensed by Animal Ethics Committee of Anhui Medical University.)

Animal experiment

16 healthy experimental canines were preoperatively given desinsectization, quarantine, numbering, weighing and fasting, but can free access to water. They were intraperitoneally sedated with 30 mg/kg of 3% pentobarbital sodium (Rongbai Biotechnology Co., Ltd., Shanghai, China). The operation steps was in the following: first, insert into the abdomen from the upper median abdominal incision to the para-aortic abdominalis, implant 100 mg sustained-release 5-Fu at single point under direct vision, and then infuse the marker stained by methylene blue, at last, implant 100 mg sustained-release 5-Fu into the left and right peritoneum about 5 cm to the incision and close the abdomen (Figure 1A~1B).

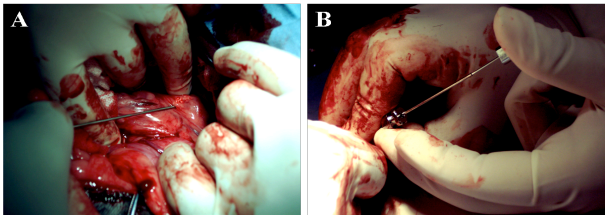

Figure 1. (A) Sustained-released 5-Fu Implantation in Peritoneum; (B) Sustained-released 5-Fu Implantation in Para-aortic Abdominalis

On days 3, 5, 7, and 10 after sustained-release 5-Fu implantation, the local implanted part was observed to check if there was congestion, adhesion, infection and necrosis. Lymphatic tissues of para-aortic abdominalis at different distances away from the implanted site, lymphatic tissue from the left subclavian and peritoneal tissue within 0~5 cm of the implanted peritoneal site were taken, and meanwhile the peripheral and portal venous blood samples were also taken. High performance liquid chromatography (HPLC) was applied to detect the above drug concentrations. These canines were finally killed according to the relevant rules of experimental animals.

Biological sample test

The steps of sample test were in the following: (1) Take the proper amount of tissue samples, cut up into pieces, put 0.5 g into 10 mL centrifuge tube with stopper (Shanghai Medical University Chemical Instrument Factory, Shanghai, China), and 0.20 mL of 500 μg/mL 5-Bru solution (Sigma chemical, Perth, Australia); (2) add 1.0 mL of 0.10 mol/L NaOH solution (MERYER CO.,LTD, Shanghai, China), then add it again after mixing with PT-MR2100 homogenizer (POLYTRON, Switzerland), and mix it with XW-80A vortex mixer (Shanghai Medical Chemistry Instrument Factory, Shanghai, China); (3) centrifuge with LXJ-II centrifugation precipitation machine (Shanghai Medical University Chemical Instrument Factory, Shanghai, China) in 3 000 r/min for 30 min, take 0.50 mL supernatant and put it into another 10 mL test tube with stopper; (4) add 0.10 g ammonium sulfate (MERYER CO., LTD, Shanghai, China) into the supernatant and mix it with vortex mixer, then transfer the supernatant to another test tube after adding 4.0 mL ethyl acetate (MERYER CO., LTD, Shanghai, China) to spin for 3 min and centrifuge for 10 min (3 000 r/min); (5) extract the residue again with 4.0 mL ethyl acetate according the previous operations, then combine the two-extracted liquid; (6) drain the liquid with vacuum dryer (temperature ≤30°C), obtain the sample residue; (7) filter the residue through 0.22 μm membrane after dissolve it into 100 μL mobile phase, and obtain the sample solution; (8) inject 20 μL sample solution in P200-II high performance liquid chromatography (Elite Scientific Instrument Co., Ltd., Dalian, China) with 20 μL quantitative loop, and record the chromatogram; (9) with 5-Fu peak area ratio on the chromatogram, calculate 5-Fu concentration in the samples according to standard curve regression equation.

Statistical data analysis

Statistical software SPSS 10.0 was used to analyse the statistics, and all the data was expressed by mean ± standard deviation (x±s). The mean between groups was compared with t test, while that among groups with analysis of variance. $P<0.05$ was considered statistically significant.

Results

General observation

During the experimental observation period, the vital signs were stable; there was no systemic reaction and

Table 1. Drug Concentration of Peritoneal Tissue at the Implanted Site (μg/g)

Time	n	Distance from the implanted site (cm)					
		0	1	2	3	4	5
3 d	4	52.53±35.20	15.12±11.54	5.32±4.31	3.48±2.74	2.33±2.21	0.92±0.73
5 d	4	46.93±37.23	10.40±8.81	3.50±2.73	2.80±2.19	1.82±1.57	0.71±0.62
7 d	4	42.64±28.83	9.64±7.63	2.64±1.94	2.29±1.63	1.48±1.30	0.64±0.44
10 d	4	36.54±32.31	5.12±3.40	1.83±1.30	1.71±0.55	0.57±0.22	0.24±0.13

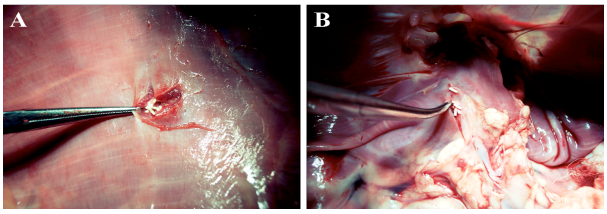

Figure 2. (A) Tissue Reaction around the Implanted Point in Peritoneum; (B) Tissue Reaction around the Implanted Point in Para-aortic Abdominalis

toxicity such as obvious anorexia, poor appetite, nausea, vomiting, aversion to cold and fever. The subjects were normal in activity, sleep and excretion, and there were no significant changes in body weight.

Damage at the implanted point

The tissues around the implanted point appeared inflammatory redness and swelling. Pathological examination showed that there was little acute and chronic inflammatory cell infiltration, but no significant cell necrosis (Figure 2A~2B).

Drug concentration of the implanted tissue

The drug concentration of peritoneum after sustained-release 5-Fu implantation: After implanted sustained-release 5-Fu, the drug concentration decreased gradually within 0~5 cm of the implanted point with the increased distance from the implanted site (P<0.05); The drug concentrations at different points also decreased gradually with time prolonging (P<0.05), but were all higher than the tumour-inhibiting concentration within 10 days of observation (the lowest tumour-inhibiting concentration was 0.05 μg/g in the tissue) (Table 1).

The drug concentration of para-aortic abdominalis after sustained-release 5-Fu implantation: After implanted sustained-release 5-Fu, the drug concentration of lymph nodes in para-aortic abdominalis decreased gradually at different distances from the implanted site with the increased distance from the implanted site (P<0.05); the drug concentrations at different points also decreases gradually with time prolonging (P<0.05), but were all higher than the tumour-inhibiting concentration within 10 days of observation (the lowest tumour-inhibiting concentration was 0.05 μg/g in the tissue) (Table 2).

Blood concentration after sustained-release 5-Fu implantation: After implanted sustained-release 5-Fu, the peripheral blood concentration was relatively low, and was not detected out after 5 days. However, the blood concentration in portal vein was about 2.5~3.75 times of that in peripheral vein, and gradually reduced with time prolonging (P<0.05).The drug concentration in portal vein was still higher than the lowest tumour-inhibiting concentration (0.1 μg/g) in 10 days (Table 3).

Table 2. Drug Concentration of Lymphatic Tissue in Para-aortic Abdominalis (μg/g)

Time	n	Distance from the implanted site (cm)			
		0	3	5	10
3 d	4	48.5±42.2	6.3±4.3	4.3±3.2	1.3±0.6
5 d	4	46.9±36.3	5.5±3.7	2.8±0.8	1.0±0.8
7 d	4	41.6±32.8	3.6±1.9	1.5±1.1	0.8±0.7
10 d	4	38.5±24.3	2.0±1.7	0.8±0.2	0.4±0.3

Table 3. Blood Concentration after Sustained-release 5-Fu Implantation (μg/mL)

Parts	n	Time (days)			
		3	5	7	10
Peripheral blood	4	0.04±0.01	0.02±0.01	no result	no result
Portal venous blood	4	0.65±0.17	0.37±0.13	0.24±0.07	0.11±0.04

Discussion

The postoperative recurrence rate of gastrointestinal tumours is very high, even more than 50%, especially gastric cancer, which is mainly due to the presence of postoperative peritoneal micrometastasis (Zhang et al., 2010; Zare et al., 2013). The latest research demonstrates that after primary lesion resection, the residual cancer cells are most sensitive to intraperitoneal chemotherapy in 7 days, and hence, it is the best time to kill residual cancer cells and micrometastases (Yamamoto et al., 2004). 7 days after the surgery, the patients are improper to receive the peripheral venous chemotherapy with severe systemic reactions due to poor constitution and incompletely-healed incision. In addition, systemic chemotherapy is not an effective option because of its low drug concentration at the tumor site and short action duration. However, peritoneally-implanted sustained-release drugs have the following characteristics: (1) long duration. It can continuously act for several days, months, even several years; (2) slowly-controlled release. The slowly-controlled release of drugs is at level 0 or 1; (3) target administration. The drug concentrations can reach the highest in the targeted area of lesions, but the total dosage is minimal to reduce the systemic toxicity; (4) loss reduction. It can effectively make use of drugs to avoid hepatic first-pass effect (Nordlinger et al., 2005; Oshima et al., 2013; Orii et al., 2013). Therefore, early application of locally intraperitoneal sustained-release chemotherapy can keep a higher drug concentration and longer action duration, consequently achieving the targeted administration and reducing systemic toxicity.

The killing effect of most chemotherapy drugs depends on time and concentration within a certain range, whereas the efficiency of intraperitoneal chemotherapy drugs not only depends on the drug concentration, but also the action duration. In conventional chemotherapy, the aqueous solvent of the drug has a shorter action duration in intraperitoneum, usually 6~8 h. In addition, the depth of chemotherapy drugs penetrating into tumours is limited from the surface. The effect of chemotherapy is greatly affected when the tumour is more than 0.5 cm (Halhsscy et al., 1994; Wan et al., 2012). However, with a good penetration in the interstitial space and cell membrane, and a certain affinity with tumour tissue, 5-Fu usually enters into the cells through passive diffusion according to a concentration gradient, and can form a higher drug concentration locally and maintain a long time (Huang et al., 2013). The literature has revealed that 5-Fu wrapped with adjuvant has a good effect in intraperitoneal chemotherapy of gastric cancer (Yoshimura et al., 2011). Besides, the efficacy of 5-Fu is positively associated with its action duration (Hu et al., 2013; Yoney et al., 2013). In general, the release time of 5-Fu wrapped with adjuvant is not more than 1 day, whereas that of sustained-release 5-Fu implantation is more than 10 days, which overcomes the disadvantage of conventional chemotherapeutic drugs with short action duration (Yao et al., 2013; Zhang et al., 2013) . Such preparations can also be easily placed at any site of residual tumours during surgery and stably maintain a higher drug concentrations at the administered area for a long time, being conductive to killing the residual tumours not removed during surgery, micrometastases and intraperitoneal free cancer cells. In addition, the local administration makes the systemic normal cells have less load, thus the toxicity of anticancer drugs is controlled to a minimum. Therefore, sustained-release 5-Fu implantation is an ideal local intraperitoneal chemotherapy preparation because of sustained-release and targeted dual properties.

Numerous clinical data indicates that the subclinical implanted metastasis formed by invasion of tumours into serosa or implantation of exfoliative cancer cells into the abdominal cavity due to surgery is the leading cause of postoperative local recurrence and liver metastasis in portal system in malignant tumours of abdominal cavity, especially progressive gastric cancer (Alexander et al., 2013). Study has found that the detection rate of peritoneal free cancerous cells comes up to 43%~55% in patients with gastric cancer (Davis et al., 2011). Both surgical procedure and stimulation can increase the exfoliation of cancer cells, and metastasis of peritoneal exfoliated cells will directly lead to the recurrence and death in patients after radical resections for gastric cancer and very low 5-year survival rate in patients with progressive gastric cancer. The most common malignant tumours in the abdominal cavity easily occur peritoneal metastasis and liver metastasis, whereas the postoperative conventional treatment including systemic intravenous chemotherapy has very low efficiency (Jarnagin et al., 2009). Currently, intraperitoneally implantation of sustained-release 5-Fu has been widely used in clinic, but its efficacy and safety are still controversial (Chen et al., 2006; Pohlen et al., 2007). The implanted sustained-release 5-Fu used in this study is the first long-term sustained release implant in China, whose active ingredient is 5-Fu. As a new sustained-release implant organically combining high-molecular polymers with good histocompatibility and commonly-used drugs recorded in pharmacopeia, it can alter the pharmacokinetics and route of administration, characterized by solid implants, intraperitoneally controlled release, long release time and locally targeted administration (Link et al., 1997). In this study, the drug concentration at the local implanted site, action duration and the reactions of the surrounding tissue were tested by using sustained-release 5-Fu implantation in the canine para-aortic abdominalis and peritoneum, which provides theoretical evidences for the prevention and treatment of peritoneal and liver metastases in intraperitoneal malignant tumours and further application of sustained-release 5-Fu in clinic.

This research results revealed that the tumour-inhibiting concentration maintained relatively high at the local implanted site in peritoneum and lymph node area of para-aortic abdominalis. Although the drug concentration diminished with increased distance away from the implanted site, but 10 days after implantation, the drug concentration at 5 cm away from the implanted site was still higher than the lowest tumour-inhibiting concentration (0.05 μg/g) (Wang et al., 2012). The results also showed that there was a higher drug concentration in the portal vein 10 days after implantation (the lowest tumour-inhibiting concentration is 0.1 μg/g in the blood) (Kirchhoff et al., 2005), suggesting that it is of great importance for the prevention and treatment of liver metastasis. Besides, there was no visible necrosis at the local implanted tissue. Hence, it can be concluded that intraperitoneal implantation of sustained-release 5-Fu is a safe and effective treatment for intraperitoneal malignant tumours, which will have good prospects for clinical application in the future.

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