RESEARCH ARTICLE

More Reasonable Animal Model for Study the Effect of Pneumoperitoneum on Abdominal Tumor Cells

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Abstract

Background: Many animal experimental studies showed that abdominal tumor cells will be widely spread during laparoscopic treatment and grow into metastases. These results are different from clinical observations. There is a hypothesis that too much tumor cells was injected in the animals lead to the results of theses bias. We aim to learn the difference of abdominal cavity volume between human body and the nude mice and to determine reasonable amount of tumor cells in the animal experiments. **Methods:** The insufflated CO_2 volume which represents the capacity of the abdominal cavity was recorded during laparoscopic process in 212 patients and 20 nude mice respectively, the relative volume of nude mice and human body was calculated.Based on data from the literature and this study , the amount of tumor cells in the animal experiments was determined. According to these data, we set up a new animal model and a traditional one respectively,and compared the rate of successful modeling and tumor formation between two animal models. **Results:** The intraperitoneal volumes of humans and nude mice were 3.01±0.36 L and 0.011±0.001 L respectively.The number of tumor cells that be uesd in animal should be approximately $0.26×10^5$ in terms of known data in human beings.Compared with the traditional animal model which formed a large number of intraperitoneal tumor metastasis, the new animal model was shows more moderately,and the rate of successful modeling was similar. **Conclusion:** In animal experiments, to simulate the clinical situation, about $0.26×10^5$ tumor cells should be inject in peritoneal cavity of the nude mice.

Keywords: Human body- pneumoperitoneum- tumor cells- animal models

Introduction

Laparoscopy has evolved dramatically over the past two decades and become integral part of treatment for digestive and gynecological benign diseases. However, the occurrence of metastatic tumor growth has precluded its widespread use for intraabdominal cancer (Nduka et al., 1994; Stocchi and Nelson, 2000). The mechanism of abdominal cancer cell spread during laparoscopic procedures for malignant disease is not well understood. But the diffusion of free tumor cells in the abdominal cavity was believed to play an important role (Allardyce et al., 1997; Wu et al.,1998).

The cell-seeding or suspension model proved a valid and probably the most common animal model when assess the safety and efficacy of laparoscopy (Yan et al., 2012; Amin et al., 2010). However,the discrepancy between experimental results and clinical observations is puzzling. Clinic data have shown optimistica outcomes of laparoscopic procedures (Bilimoria et al.,2008; Jayne et al., 2007; Li et al., 2014), but animal experiment have shown more aggressive metastatic tumor spread compared with conventional surgery (Gitzelmann et al., 2000). The effectiveness of this model in simulating clinical practice worth to discuss.The high incidence of tumor masses and metastases in these animal experiments are hardly to compare with clinical findings. Many studies have proved that the amount of tumor cell inoculum can affect the outcomes (Allardyce et al., 1997; Wu et al., 1998). Umpleby found out that the median tumor cells in lavage fluid from patients who have performed colon cancer resection is $0.78×10^6$. But, the great majority of animal models have used various amount of tumor cells ranging from $1×10^5$ to $1×10^8$, without any standard . The number of tumor cell was undoubtedly too large for small animals, and it might result in an exaggerated response (Umpleby et al.,1984). To determine the reasonable injection quantity of tumor cells has important significance on the establishment of animal model which is consistent with the clinical situation.In the experiment, the density of abdominal tumor cell was affected by the number of tumor cells and the volume of abdominal cavity. Thus, This study mainly aim to learn the difference of abdominal cavity volume between human body and the nude mice to

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determine a reasonable amount of tumor cells in the animal experiments.To built a more reasonable animal model to study the effect of pneumoperitoneum of abdominal tumor .

Materials and Methods

Clinical Experiment

From June 2011 to December 2014, 212 patients undergoing laparoscopic procedures were enrolled in the study. Indications for surgery were: gastric cancer, colorectal Cancer (Table 1). The experimental protocol was approved by the Ethical Committee of the North Sichuan Medical College, China.

All patients were submitted to general endotracheal anesthesia and muscle relaxants with supine posture. The reusable Veress was inserted into the abdomen under continuous flow of CO_2 at least 1 L/minute. Pneumoperitoneum was established and the insufflated CO_2 volume was recorded when the intraabdominal pressure was steadily at 15 mmHg.

Animal Experiment

20 Nude mice aged 9 to 10 weeks (mean weight 20.55g) were selected. All animals were kept under standard laboratory conditions(temperature, 20~24 ºC; relative humidity, 50%~60%;12hours of daylight, 12 hours of darkness), fed with laboratory diet and allowed drinking water freely.

Each nude mice was anesthetized by pentobarbital sodium then restrained in the supine position .Skin disinfection by 75% alcohol, then the abdominal wall of the nude mouse was raised, and a 18-G needle was inserted into the left lower abdomen.The needle was connected with the inflator with flow meter which was used to record the volume of CO_2 .The process of inflated gas was discontinued when the abdominal pressure was maintained at 5 mmHg and the insufflated CO_2 volume was recorded. Based on data from the literature and the relative volume of nude mice and human body , the amount of tumor cells in the animal experiments was determined.Then we establish animal model of 20 nude mice with Gastric cancer cell line SGC7901.Among them, the amount of tumor cells were injected in 10 nude mice (Group A) was based on the research results,And the other 10 nude mice (Group B) were injected with 1×10^6 cancer cell according to the literatures .These nude mice were killed by an overdose

of anesthetic 14 days after being injected with tumor cells. According to the semiquantitative Peritoneal Metastatic Score (SPMS) (Kinugasa et al.,2004), the intraperitoneal nodules distribution, quantity, average diameter, ascites and distant metastasis of nude mice were compared between the two groups. The abdominal wall was divided into three sectors(left abdominal wall,right abdominal wall,and the surface of the organs) for the purpose of scoring tumor implantation and growth (Table 2) .For each sector, the grade for the number of tumor nodules was multiplied by the grade for the average size of the nodules. A SPMS score was then calculated by summing the scores for each of the 3 sectors, to give a score in the range of 0–27. The independent samples t test was used to compare the differences outcome between the two groups.

Results

The mean (±SD) of intraperitoneal volumes of humans and nude mice were 3.01± 0.36 L and 0.011±0.001 L respectively. The intraperitoneal volume of the humans is approximately 300 times bigger than that of the nude rats during laparoscopy. The median of free cancer cells in
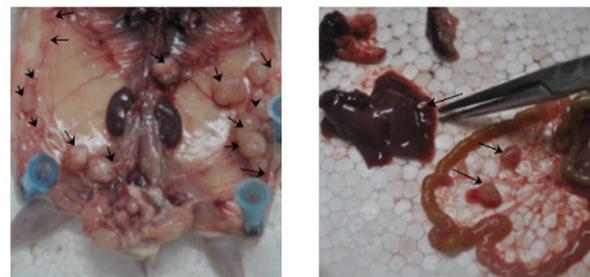

Figure 2. Peritoneal Tumor Metastasis in Nude Mice of Group B. * → indicates tumor nodules

Table 1. The Basic Information of Patients

	range/case number	mean/ratio
Gender		
Male	116	55%
Female	96	45%
Age (years)	28~80	mean=60.7
BMI* (kg/m²)	14.5-36.7	mean=23.2
Types of diseases		
Colorectal ancer	123	58%
Gastric cancer	89	42%

* BMI, Body Mass Index

Table 2. The SPMS for Evaluate Peritoneum Nodules

number of tumor nodules	score	mean of the maximum diameter of tumor nodules	score
none	0		
3 or less	1	<2 mm	1
moderate tumor growth	2	2–5 mm	2
abundant or confluent tumor	3	>5 mm	3

Figure 1. Peritoneal Tumor Metastasis in Nude Mice of Group A. *→ indicates tumor nodules

patients' peritoneal cavity was $0.78×10^6$. So,The number of tumor cells injected into peritoneal cavity in the nude mice model should be $0.26×105$ in terms of known data in humans.Based on these data, we establish animal model with $0.26×105$ gastric cancer cell inject in the abdominal cavity of nude mouse in group A.Correspondingly, $1×10^6$ gastric cancer cell were injected for each mouse in group B.14 days later,eight nude mice of group A had a large number of tumor nodules on peritoneum (Figure 1), and the other two had few nodule on peritoneum .In group B,nine nude mice had a great large number of tumor nodules on peritoneum (Figure 2), the other one also has a few nodule on peritoneum.The probability of establishing a satisfactory animal model was 80% vs 90% in two groups.The SPMS score of intraperitoneal tumor nodules were 10.2±5.0 and 19.1±6.9 respectively, and there was significant differ-ences between the two groups, (*Independent-samples T test, p=0.004*).

Discussion

Laparoscopic surgery has opened a new era in modern surgery. But it's role in the treatment of malignant tumors has been controversial, only some of the qualified medical centers are used it in clinical researches. Some basic research showed that CO_2 pneumoperitoneum may promote the proliferation of tumour cells in the abdominal cavity (Nduka et al., 1994; Stocchi and Nelson, 2000; Ost et al., 2008; Nakada et al., 2005). Disseminated forms mainly include the metastasis of tumor cells grown in the port-site and the abdominal cavity.The incidence of port-site metastases after laparoscopic resection of various malignancies was 0% ~ 21% (Reymond et al., 1998). Although the incidence of implantation metastasis in abdominal cavity is not very clear,free tumor cells in the abdominal cavity are considered main cause of peritoneal dissemination (Carmignani and Sugarbaker 2004). But the clinical observation results are far more exciting. Many clinical retrospective studies and several recently published randomized controlled trials have considered that, compared with open surgery, laparoscopic does not increase the proliferation and metastasis of abdominal tumor (Roviello et al., 2015; Son et al., 2014; Sakuramoto et al., 2013; Tanaka et al.,2014). What is the reason for these different results? The animal model used in these experiments is to inject suspension which contains about $1×10^5 ~ 1× 10^8$ free tumor cells into the abdominal cavity. However,previous studies have demonstrated the number of injected tumor cells influence the cell survival, as well as the impact on the tumor weight and development of metastsses (Allardyce et al., 1997; Wu et al., 1998), The intraperitoneal free tumor cells might play a crucial role in the formation of metastasis and peritoneal dissemination. The number of tumor cells in the peritoneal cavity is probably one of the most important factors affecting the results of these basic and clinical researches.Thus, we believe that these different results obtained from basic experimental and clinical studies may be related to the irrational animal model.In order to get the density of free tumor cells in the abdominal cavity of human, we measure

the volume of patient and the number of tumor cells in their abdominal cavity. Accordingly, the volume of abdominal cavity in nude mice was also measured.Animal models with the same density of tumor cells in abdominal cavity can be established on the basis of comparative differences between human and animals.

Human abdominal cavity volume has seldom been reported.A previous study (Abu-Rafea et al., 2006) has shown that when the abdominal pressure is increased from 0 to 15 mm Hg abdominal volume of human body was increased linearly. However, when the abdominal pressure continues to rise from 15 to 30 mmHg,the abdominal cavity volume did not change significantly.And in laparoscopic surgery, the pneumoperitoneum pressure is set to 15 mmHg can get a good view and less cardiopulmonary complications.Therefore, we set 15 mmHg for human experiment in the present study. And we obtained data of human abdominal cavity volume by measuring the volume of CO_2 flow in 212 patients who received laparoscopic surgery.Although not fully reflect the human abdominal volume under physiological conditions, this data was very valuable for the study of distribution of free intraperitoneal tumor cell in laparoscopic process. Our data showed that the intraperitoneal volumes of humans was 3.01±0.36 L during laparoscopic access.that is consistent with the literature report (Abu-Rafea et al., 2006).

At the same time, it is very important to accurately measure the volume of the abdominal cavity of nude mice under the condition of laparoscopy.Now there is no relevant reports in the literature.In the experiment of laparoscopic surgery in nude mice, often setting pneumoperitoneum pressure for 5 mmHg,it was the maximum pressure that nude mice can tolerate long time. So we set 5 mmHg for animal experiment in the present study.The intraperitoneal volumes of nude mice was 0.01±0.001 L during laparoscopic access.

These results imply that the intraperitoneal volume of the humans is approximately 300 times bigger than that of the nude mice during laparoscopy. According to the study of Umpleby, the median of free tumor cells in ab-dominal cavity from patients who have performed colon cancer resection is $0.78×10^6$ (Umpleby et al., 1984), thus the amount of tumor cell employed in the nude mice model should be $0.26×105$. Based on the data from this study and previous literatures, we established two different animal models.The results showed that the animal model can also be successfully established with injected fewer tumor cells according to the human body data. And the animal model established based on the data that obtained from the preliminary clinical and animal experiments is more close to the clinical situation.Thus, the bias that caused by use of too many tumor cells in experiment can be avoid.

Currently, there is no uniform standard for the injection of tumor cells in animal experiment, this study may have a positive effect on formulate this standard.And we can also determine the amount of tumor cell inoculum according to different animal model if we know their abdominal capacity .The deficiency of this study is only to select the data from patient with gastric cancer and colorectal

cancer.Further research is required for other types of abdominal tumors.

In conclusion, based on the difference of the intraperitoneal volume between nude mice and human beings during laparoscopy, we believe that in order to simulate clinical situation, the amount of tumor cell suspension injected in the abdominal cavity of nude mice should be 0.26×105.

Acknowledgments

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