Study on Radiation Injury Induced by Covered Metal Stent with ^{125}I Seeds in Normal Rabbit Esophagus

Sun Changbo[1] Lv Bin[1] Bao Haibiao[1] Ni Guibao[2] Ren Yongbao[2]

1 Department of Gastroenterology,the First Affiliated Hospital of Zhejiang Chinese Medical University,Hangzhou,310006

2 Department of Pathology,the First Affiliated Hospital of Zhejiang Chinese Medical University,Hangzhou,310006

【Abstract】

Objective — To evaluate the tissue response to brachytherapy of the radioactive metallic stent loaded with ^{125}I seeds in normal rabbit esophagus.

Methods — A specially designed self-expandable covered metallic stent preloaded with ^{125}I seeds in sheaths was developed. 12 rabbits were randomly assigned to 2 different groups. Test group was implanted with stents loaded with ^{125}I seeds（22.2MBq× 3 seeds，n=6），control group with stents without ^{125}I seeds (n=6). The rabbits were killed in the 2nd, 4th and 8th week after implantation of the stents following esophagus X-ray examination to observe migration of stents and ^{125}I seeds. Morphological observation and histological examination of the esophageal wall were performed.

Results — No ^{125}I seed migration was found during the implant of stent and follow-up. There was no perforation of esophagus 2 weeks after implantation of the stents, the esophagus tissue in the middle part of the stent in trail group, slight epithelial hyperplasia on the stent surface and submucosal inflammatory cell were observed. In the 4th week, hyperplasia of granulation and slight fibrosis were observed. In the 8th week, fibrosis tissue was more significant. The injury of esophageal tissue opposite the ^{125}I seed was significantly milder than the tissue touched with ^{125}I seed. In control group, the pathological change of esophagus tissue was similar to normal tissue, slight epithelial hyperplasia was observed. However, at both ends of the stent, the severe local hyperplasia of esophagus tissue covered the stent, microscopically, severe granulation tissue hyperplasia and fibrosis could be observed in both groups.

Conclusion — The main pathological change of esophagus wall adjacent to the ^{125}I seed was granulation tissue hyperplasia and fibrosis. No bleeding or perforation was found. ^{125}I seeds-loaded mental stents can be used in normal rabbit.

【Key words】 Esophagus; Stent; ^{125}I seed; Brachytherapy; Radiation Injury; Rabbit

The esophageal cancer is a common malignant disease in China. 80% of the patients are already in middle/late stage when they are diagnosed. [1] Therefore only the palliative therapies are applied. In recent years, the therapy of implanting metallic stents has been applied in palliate malignant esophageal cancer. This therapy effectively released the symptoms of dysphagia and improved the quality of patients' lives. The therapy of implanting radioactive seeds has been applied in treating malignant tumors like adenoma of prostate and glioblastoma overseas. [2, 3] The ^{125}I Radioactive Covered Stent may locally control the tumor and slow down the growth of tumor tissue around stent. This experiment discussed the histopathological changes of normal esophagus and provided the experimental basis for the therapy of implanting the ^{125}I Radioactive Covered Stent in the treatment of malignant esophageal stricture by implanting the ^{125}I

Radioactive Covered Stent into normal rabbit esophagus.

Material and Method

1. Material

1) Experimental animal: Bought from The Laboratory Animal Center of Zhejiang Chinese Medical University. The pure breed New Zealand Rabbits aged 4-5 months and weighted (2.5±0.5) kg of each gender.
2) Covered Nitinol Esophageal Stent: Provided by Micro-Tech (Nanjing) Co.,Ltd. The stents were cylinder-shaped, 20mm in length and10mm in diameter. The diameter of nitinol wires was 0.16mm.
3) ^{125}I seeds: Bought from Ningbo Junan Pharmaceutical Technology Co., Ltd. The seeds were Cylinder-shaped, 4.5mm in length and 0.8mm in diameter. Their half lives are 59.6 days and their surface intensity of radioactivity is 22.2MBq. They emitted 27.4Kev X-ray, 31.4Kev X-ray or 35.5Kev γ-ray.

2. Methods

1. Loading the ^{125}I seeds: we use the esophageal stents for laboratory animals as the carriers of the internal radiation seeds. According to the results of the experiments, the lengths suitable for laboratory animals were 20mm and 10mm in diameter.. Each fixed sheath that being regularly sewed onto the outer surface of stent should exactly be loaded with one ^{125}I seed. One fixing sheath was in the middle of the stent and there were sheaths 6mm apart from it in different directions.
2. The animal experiment of internal radiation esophageal stent: 12 healthy rabbits were divided into 2 groups-the test group (each was implanted with stent loading 3 seeds, the dosage of each seed was 22.2MBq) and the control group (each was implanted with normal laboratory animal esophageal stent). There were 6 rabbits in each group. After intravenous anesthesia from ears with 3% pentobarbital at a dose of 1ml/kg, the rabbits were fixed in the DSA operating tables. Then the operator radiographically deployed the stent until the proximal end of stents reached the rabbits' bifurcation of trachea.
3. Visual observation: 2,4,8 weeks after operation, we separately took out 2 rabbits and killed them by intravenous injection of 10 ml air from ears. Then we incised their chests at once and observed their extents of esophageal damages near the stents.
4. Observation under light microscope: The esophageal tissue in a radius of 20mm away from the radioactive seeds was biopsied and then fixed, embedded, sliced and HE dyed. Two experts from Pathology Department inspected the tissues under microscope and graded them according to the extents of damages. [4]Mucous membrane damages were divided into +, ++ and +++, separately marked as 2/3, 4/3 and 2 points. Submucosal changes were also divided into +, ++ and +++, based on .haemorrhage, inflammatory infiltration and necrosis separately given 1/3, 2/3 and 1 points. Tunica adventitia and muscular layer were also divided into +, ++ and +++, separately marked as 1, 2 and 3 points. Normal control was marked as 0 point.

Results

1. The situation of ^{125}I radioactive stents

All stents were smoothly deployed in the exact position and there was no seed falling off. There were no related complications or deaths. At the end of the experiment, ^{125}I seeds were still being fixed in the sheath without falling.

2. The general morphological changes

2, 4, 8 weeks after operation, we separately took 2 rabbits from the experimental group and the control group, anatomized their esophagus to observe them. The stent migration for about 22mm towards the esophageal distal end happened to one rabbit after 4-8 weeks follow-up. But the stent did not reach the gastric cavity. The other stents did not migrate. 2 weeks after operation, we retrieved the stents in experimental group and control group, and then found the stents were clear with few food residues. There was a few esophageal hyperblastosis at the ends the stents and the stents could be easily separated from the esophageal inner membranes. 4 weeks after operation, there was obvious proliferation at the ends the stents, the stents were clear and ends of the lumen were strictured. The food residues were cloged in the stents. 8 weeks after operation, the esophageal walls were getting thicker and bulged into the esophagus. The stents could not be easily separated from the esophageal walls. 2, 4, 8 weeks after operation, there were no tissues growing into the covered stent.

3. Pathologic changes

3.1 The esophageal tissues in middle part of stents

2 weeks after operation, there was esophageal squamous epithelium hyperplasia in the experimental group, the cell alleys were disorganized. The expansion and hyperaemia of capillary vessels could be seen in submucosal layer. Inflammatory cell infiltration was also observed. Being graded according to the extents of damages, their values were 1.15±0.21. 4 weeks after

operation, the mucous membrane-erosion and necrosis could be seen in the experimental group. And the glands markedly reduced while the inflammatory cells distinctly increased. Granulation tissue hyperplasias and few fibrous tissue hyperplasias were seen. They were valued as 2.0 points according to their degree of injury. 8 weeks after the operation, inflammatory cell infiltrations in submucosa reduced while lots of fibrous tissue hyperplasias and expansions appeared. The congestive capillary vessels disappeared on the whole. They were valued as 2.5±0.28 points according to their degree of injury. The histopathological changes in esophageal parts where 3 ^{125}I seeds located had a similarity. They appeared to be the light dysplasias in esophageal squamous epithelium and few inflammatory cell infiltrations in submucosa. 2, 4 and 8 weeks after operation, their injury severities were separately valued 0.5±0.24, 0.83±0.24, 1.17±0.24.

3.2 The esophageal tissues at the ends of stents

2 weeks after operation:, the experimental group appeared the pathologic changes as the obvious esophageal squamous epithelium hyperplasia, mucous membrane-erosions, necrosis and inflammatory cell infiltrations compared with the control group. The expansion and hyperaemia of capillary vessels as well as the granuloma hyperblastosis could be seen in submucosal layer. Their values were 2.15±0.21. 4 weeks after operation,the mucous membrane-ulcer and local lamellar necrosis could be seen. There were still a lot of inflammatory cells. The granulation tissue, fibrous connective tissue hyperblastosis and the decrease of submucosal glands markedly appeared. The proprius muscular layers thinned and the sporadic inflammatory cell infiltrations could be seen. Their injury severities were scored as 3.5±0.71. 8 weeks after the operation as compared with the situation 4 weeks after operation, the fibroplastic proliferations obviously densified while the fibroblasts, inflammatory cells and renascent capillary vessels reduced. The proprius muscular layers were still complete but were thinner than 4 weeks ago. Their injury severities were valued 4.5±0.71. As the implantation time prolonged, the injury severity values added. In the experimental group, the injury severity values in esophageal parts which contacted the seeds directly were higher than the opposite regions, but lower than the regions at the ends of stent.

Discussion

For the patients with late-stage esophageal cancer, the surgical operations have high risks and recurernce rates. And the curative effects of surgical operations were not good. EBRT (External Beam Radiation Therapy) is the main therapy for late-stage esophageal cancer but it has high complication rate as 14.9%. The complications include esophago-tracheal fistula, radiation pneumonitis, esophageal stenosis and esophageal ulcer, etc. The implantation of esophageal stent can solve the dysphagia of patients with late-stage esophageal cancer, but it doesn't have the therapeutical effects on the growth of tumors. The overgrowth of tumors may lead to the restenosis then the endoscopic therapy is needed again, it impacts the curative effect, which raises clinical problem. The ^{125}I seeds have a good curative effect for prostatic cancer and glioblastoma when they're used in inter-organization brachytherapy. The characteristics of ^{125}I seeds stents lie in that the ^{125}I seeds functions and cures in the diseased region when the stents solve the dysphagia of patients at the same time. Therefore the curative effect can be improved. Seeds with different radiological doses cause different pathological changes of esophagus. The high dose rate radiation in close quarters may cause the severe damages of esophagus. The damages appear to be lamellar necrosis of mucosa and submucosa, hardening and perforation of muscular layer. And the damages came up early and severe. Won implanted the 166Ho (half life 28.6h) Esophageal Stent into the dogs in experiments and found the high dosage group (194-383 Gy) appeared the severe fibrosis in esophagus and muscularis propria denaturalization, even the fibrosis of esophageal full-thickness appeared. In the low dosage group (23-32Gy), the glands atrophy and submucosa inflammation appeared. The ^{125}I seeds used in this experiment continuously emited Y-ray and their effective illumination diameters were 15-20mm. With the distance prolonged, the energy of ray rapidly decayed. Therefore the most dosages of ray were effective in a limited area around seed and had very little effects on the distant tissues. The distribution characteristics of these dosages were suitable for the inter-organizational radiotherapy. What's more, the energy of Y ray ranges from 27KeV to 35KeV, therefore the safety of operator has been significantly improved. As for the patients, even without special protective measures, there won't be radioactive contamination harming the persons around them,All the animals stayed alive 8 weeks after implantation of ^{125}I Seeds Radioactive

Stents. All of them took food normally and gained some weights; excepted one case was sick after meals and choked because of the stricture at the two ends of stent. Using endoscope to observe and analyze, there were only slight pathologic injuries mainly in epithelial layers in middle esophagus in the Experiment Group 2 weeks after the operation and the. The squamous epithelium markedly proliferated and the inflammatory cell infiltration appeared in submucosa. 4 weeks after the operation, the hyperplasia of granulation tissues and few fibrous tissues appeared. 8 weeks after the operation, the inflammatory cells in submucosa reduced and the fibrous connective tissues were more densed. In the Control Group, the middle part of esophagus did not change obviously along with time. The squamous epithelium had slight hyperplasia and the inflammatory cell infiltration appeared in submucosa. The experimental results showed that ^{125}I seeds damaged esophageal tissues mainly in mucosa and submucosa while the muscularis propria did not obviously change. The injury of ^{125}I seeds to normal esophageal tissues was slight, basically the same as the findings by Dr. Jinhe Guo in China, which might be associated with the low radioactive dosage of ^{125}I seeds. Therefore, the continuous intra cavitary irradiation by ^{125}I seeds of low energy and low dosage rate is different from the external beam radiotherapy by high dosage in clinical application; the former exerts light pathological injury on the surrounding tissues and will not cause the complications such as perforation and hemorrhage, etc. Because of the shearing force by esophageal peristalsis as well as the pressure at the ends of stents, the changes of esophageal tissues turned to be similar in the Experiment Group and the Control Group. The obvious pathology changes appeared 2 weeks after the operation; the esophageal mucosa, granulation tissues and fibrous connective tissues had obvious hyperplasia 8 weeks after operation. These pathologic changes are basically the same as the results by Dr. Zhaoshen Lee in China. Therefore, the radioactive stents does not have obvious effects on the proliferative tissues at both ends of the stent. In a word, the implantation of ^{125}I Seeds Covered Radioactive Stent is safe and practicable. ^{125}I seeds only pathologically injury the mucosa and submucosa. The injury mainly appeared as local inflammation, hyperplasia of granulation tissues and fibrosis. No severe injury or complications happened. This experiment provides further evidences for the researches of treating esophageal cancer by ^{125}I Seeds Covered Radioactive Stent.

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