Effect of ^{125}I seeds and ^{103}Pd stents on proliferation of vascular smooth muscle cells

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Abstract This study aims at the theoretical and practical evidence for prevention of restenosis *in vitro*. Vascular smooth muscle cell (VSMC) model was established using adherent cell culture methods. The proliferation of VSMC was investigated by the cell counting method and ^{3}H-TDR implementation test. The results are as follows. (1) For ^{125}I-seeds, the inhibition rate was 29.3% at 74 Bq (p <0.05), 35.2% at 148 Bq (p <0.05) and 42.4% at 370 Bq (p <0.05). For ^{103}Pd-implanted stents, the inhibition rate was 14.7% at 4.44 MBq (p <0.05), 24.0% at 5.92 MBq(p <0.05) and 38.0% at 7.4 MBq (p <0.05). There was no significant difference between the blank tests and non-radioactive tests. (2) 48 hours exposure from ^{125}I-seeds at 148 Bq or ^{103}Pd-implanted stents at 7.4 MBq did not result in VSMC's morphological alteration, but that from ^{125}I-seeds at 370 Bq caused morphological changes. Both ^{125}I-seeds and ^{103}Pd-implanted stents inhibit the VSMC DNA synthesis *in vitro*. The inhibition effects are significantly related to their exposure duration and doses.

Keywords ^{125}I-seeds, ^{103}Pd-implanted stents, Vascular smooth muscle cell

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1 INTRODUCTION

The percutaneous transluminal coronary angioplasty (PTCA) technique has been widely used to cure coronary heart disease and save millions of lives since 1977. However, the 30%-50% incidence of restenosis within 3-6 months after PTCA is the major barrier for its practical application. Many methods, such as new drugs and transfer gene have been clinically tested to prevent restenosis, but often proved futile. Although in recent years, the implant of radioactive stents, such as ^{32}P, ^{188}Re and ^{90}Y has been considered as an effective and feasible method, many problems are still unresolved. The main purpose of this research is to find a new solution for prevention of restenosis after PTCA and provide a theoretical and experimental evidence for clinical application. The effects of ^{125}I-seeds and ^{103}Pd-implanted stents on the vascular smooth muscle cell (VSMC) proliferation was observed and studied *in vitro*.

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2 METHODS

(1) Cultivation of VSMC

The experimental procedures were performed according to the routine method.

(2) Verification of VSMC

The morphological and immunochemistry methods were used to verify VSMC.

(3) Cell proliferation counting test

The 3rd-7th generation cells were digested by 0.125% trypsin-EDTA. Add 10% FCS M199 culture solution to terminate digestion. Count and adjust cell concentration to 4×10^7/L. Add 0.5 mL cell solution into each hole of the cell culture dishes (2×10^4 cells). Put various radioactive doses of ^{125}I-seeds or ^{103}Pd stents into the VSMC culture tubes and count the cells in 24 h, 48 h, 72 h and 96 h respectively.

(4) ^{3}H-TDR implementation tests

The experimental procedures were performed according to the routine methods.

(5) Experiment groups

Blank Control Group: 0.2%FCS M199 culture solution, without ^{125}I-seeds or ^{103}Pd stents; Non-radioactive Control Group: 1%FCS M199 or 10%FCS M199 with non-radioactive I-seeds or non-radioactive stents; Experimental Group: a) 1%FCS M199 culture solution+^{125}I-seeds or ^{103}Pd stents with various radioactivity, and b) 10%FCS M199 culture solution+^{125}I-seeds or ^{103}Pd stents with various radioactivity.

(6) Formula

Inhibition percentage=(1-Experimental Group CPM/Control Group)×100%

3 RESULTS

3.1 Verification of vascular smooth muscle cell (VSMC)

Morphological observation: Cells are in the shape of shuttle or long shuttle under microscope, piled and in wave crests and troughs. See Fig.1.

Immunochemistry verification: Positive by staining with a specific smooth muscle α-actin antibody. The nucleolus appears blue and the cytoplasm brown. See Fig.2.

3.2 ^{3}H-TDR implementation tests

(1) Effects of ^{125}I-seeds and ^{103}Pd stents on 10% fetal bovine serum (FCS)-induced VSMC DNA synthesis.

In ^{125}I-seeds, 148 Bq and 370 Bq can inhibit 10% FCS-induced VSMC DNA synthesis markedly, among them the inhibition rate of 148 Bq ^{125}I-seeds is 20.6% (p <0.01); whereas that of 370 Bq is 44.2% (p <0.001). In low radioactive ^{125}I-seeds (18.5–74 Bq) there is no distinct influence on 10% FCS-induced VSMC DNA synthesis (p > 0.05). Statistically,

Blank group and non-radioactive seeds group have no significant difference ($p > 0.05$). See Fig.3.

Fig.1 Cell's morphology

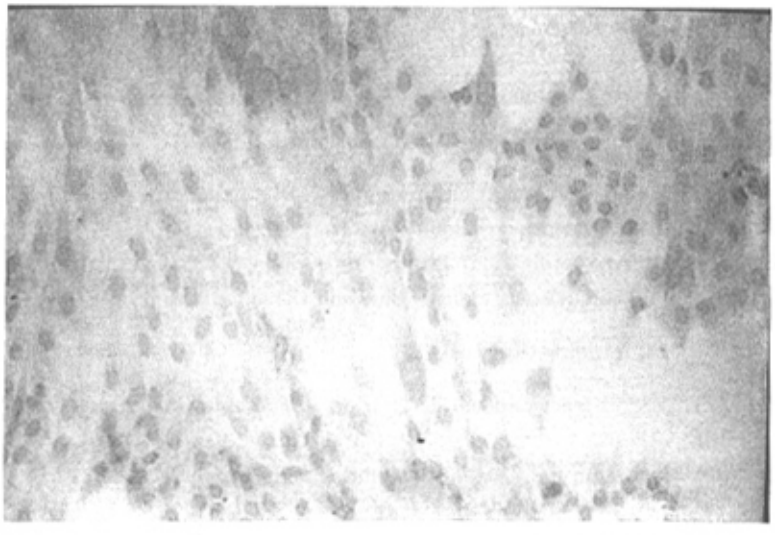

Fig.2 VSM cell's immunochemistry verification

With regard to ^{103}Pd stents, 4.44–7.4 MBq can significantly inhibit 10% FCS-induced VSMC DNA synthesis. The inhibition rate of ^{103}Pd stents of 4.44 MBq, 5.92MBq and 7.4MBq is 18.3% (p <0.01), 28.5% (p <0.01), and 40.8% (p <0.001), respectively. In low radioactive ^{103}Pd stents (1.48–2.96 MBq) there is no distinct influence on 10% FCS-induced VSMC DNA synthesis (p >0.05). Statistically, blank group and non-radioactive seeds group have no significant difference (p >0.05). See Fig.4.

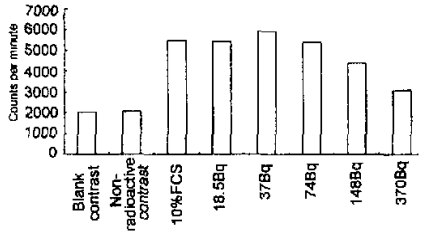

Fig.3 Effects of ^{125}I-seeds on 10% FCS-induced VSMC DNA synthesis with various radioactivity

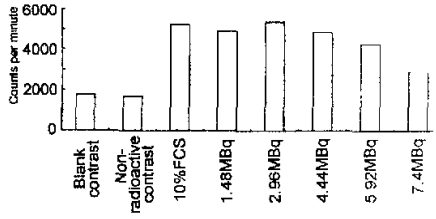

Fig.4 Effects of ^{103}Pd-stents on 10% FCS-induced VSMC DNA synthesis with various radioactivity

(2) The effects of ^{125}I-seeds and ^{103}Pd stents on VSMC DNA synthesis without fetal bovine serum (FCS) inducement.

In ^{125}I-seeds, 74–370 Bq can inhibit VSMC DNA synthesis markedly without 10% FCS-induced. The inhibition rate of 74 Bq, 148 Bq and 370 Bq ^{125}I-seeds is 29.3% (p <0.05), 35.2% (p <0.05), and 44.2% (p <0.001), respectively. The low radioactive ^{125}I-seed (18.5–37 Bq), there is no distinct influence on VSMC DNA synthesis (p >0.05). Statistically, blank group and non-radioactive seeds group have no significant difference (p >0.05). See Fig.5.

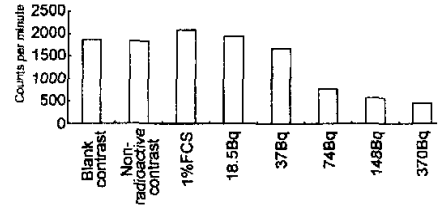

Fig.5 Effects of ^{125}I-seeds on VSMC DNA synthesis without fetal bovine serum (FCS) inducement

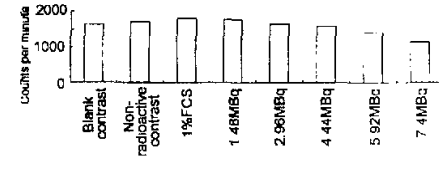

Fig.6 Effects of ^{103}Pd stents on VSMC DNA synthesis without fetal bovine serum (FCS) inducement

With regard to ^{103}Pd stents, 4.44–7.4 MBq can significantly inhibit VSMC DNA synthesis without 10% FCS-induced. The inhibition rate of 4.44 MBq, 5.92 MBq and 7.4MBq ^{103}Pd stents is 14.7% (p <0.01), 24.0% (p <0.01), and 38.0% (p <0.001), respectively. In low radioactive ^{103}Pd stents (1.48–2.96 MBq) there is no distinct influence on VSMC DNA synthesis (p >0.05). Statistically, blank group and non-radioactive seeds group had no significant difference (p >0.05). See Fig.6. Both 148–370 Bq ^{125}I-seeds and 4.44–7.4 MBq ^{103}Pd stents can inhibit VSMC 's proliferation regardless of 10% FCS inducement (p <0.05).

3.3 Cells proliferation counting

(1) Normal VSMCs proliferate rapidly, then peak in 24–72 hours, and gradually to a platform.

(2) 74 Bq ^{125}I-seeds can inhibit the proliferation of VSMC DNA synthesis after 48 hours. The effect of ^{125}I-seeds on proliferation is dependent on time, see Fig.7.

(3) 5.92 MBq ^{103}Pd stent can inhibit the proliferation of VSMC DNA synthesis after 48 hours. The effect of ^{103}Pd stents on cell's proliferation is dependent on time, see Fig.8.

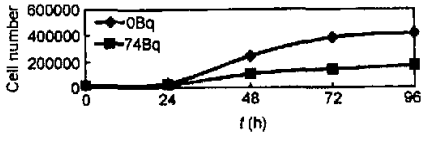

Fig.7 The 74 Bq ^{125}I-seeds' effect on cell proliferation counting at different time

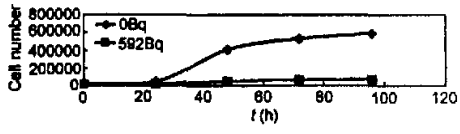

Fig.8 The 5.92 MBq ^{103}Pd stent' effect on cell proliferation counting at different time

3.4 Morphological observation

148 Bq ^{125}I-seeds or 5.92 MBq ^{103}Pd stents didn't cause cell's death and no change was observed in cell's morphology. But 370 Bq ^{125}I-seeds could cause cell's change in morphology.

4 DISCUSSION

It is well known that there are three components of restenosis:[1–3] elastic recoil, intimal proliferation, and analogue to wound contracture.

Although the adoption of inner-vascular stents can inhibit elastic recoil, prevent remodeling, and lower the incidence of restenosis,[4] the severe neointima hyperplasia of SMCs resulting in restenosis is still predominant. In animal models of balloon-induced arterial injury, neointimal hyperplasia is progressive over the first 4 to 6 weeks.[5] In

one study using a rat carotid artery-balloon injury model, maximum DNA synthesis, coincident with active intimal smooth muscle cell proliferation, was seen within the first week after balloon angioplasty. This proliferation started to decrease during the second week and decreased to control levels approximately 28 days after balloon injury.[6] These results suggest that any intervention aimed at reducing neointimal hyperplasia is most likely to have a therapeutic effect if delivered early and continuously within the first several weeks after balloon angioplasty. The animal experiments show that the radioactive stents implanted in the vessel following PTCA can inhibit proliferation and migration of SMCs. The purpose of our experiment is to observe the effects of ^{125}I-seeds and ^{103}Pd stents on VSMC's proliferation and determine the proper and ideal radionuclide dosage.

^{125}I-seeds are mainly used in treatment of prostate hyperplasia. Our research conducted on the properties of ^{125}I *in vitro* VSMC proliferation shows that 74–370 Bq ^{125}I-seeds can inhibit cells' proliferation, and thus a design of ^{125}I-stent may be practically possible in near future.

The average energy of ^{103}Pd is 21 keV, an energy suitable for inside vascular irradiation to inhibit restenosis. Also, its half life (17 days) has potential efficacy in reducing neointimal hyperplasia after balloon injury (4–6 weeks). However, its relatively low energy demands a large dosage of 7.4 MBq. Although the clinical use of ^{103}Pd-stent has already been reported, many questions are left unsolved, such as how the appropriate dosage for preventing restenosis without inducing vessel fibrosis and obliteration is determined; how the radiation inhibit restenosis effectively or only temporarily delay the occurrence of restenosis, or whether it can cause the cells apoptosis.

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