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A Novel Method of Brachytherapy Using Local Delivery of ^{99m}Tc-HMPAO for Coronary Stent Restenosis

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Background : Restenosis after percutaneous coronary intervention (PCI) is a matter that still remains to be resolved. Herein, the inhibitory effect of locally delivered ^{99m}Tc-HMPAO (hexamethyl propylene amine oxime) on neointimal hyperplasia after coronary stenting was examined in a pocine model, and its safety and efficacy observed in patients with coronary stent restenosis.

Methods : After a stent overdilation injury, local radioisotope delivery using ^{99m}Tc-HMPAO was applied to one coronary artery (Group I) and control therapy to another (Group II) in each of 10 pigs. Follow-up coronary angiogram (CAG) and histopathologic assessment were performed 4 weeks after stenting. Eleven patients (10 males and one female, 62.4±5.7 years of age) underwent local administration of 30 mCi/ 2 mL ^{99m}Tc-HMPAO shortly after PCI, via a Dispatch Catheter™, followed by a whole body scan to evaluate the distribution of the ^{99m}Tc-HMPAO, as well as a thallium-201 (Tl-201) myocardial scan to evaluate myocardial perfusion. The major adverse cardiac events (MACE) were assessed during a one-year clinical follow-up.

Results : On histopathological analysis, the neointimal areas were 1.2±0.6 and 2.7±0.4 mm^2 (p=0.002), and the histopathological areas of stenosis were 27.16.3 and 53.4±5.2% in Groups I and II (p=0.001), respectively. In the clinical study, there was no in-hospital MACE. On a quantitative coronary angiographic analysis, the minimal luminal diameter was increased from 0.4±0.3 to 2.9±0.2 mm, and diameter stenosis decreased from 84.2±9.5 to 16.3±11.0% following PCI. Follow-up CAG was performed in 9 cases (81.8%) and restenosis occurred in 2 (22.2%). On a follow-up CAG, the minimal luminal diameter, diameter stenosis rate, lumen loss and loss index were 2.0±0.8 mm, 27.7±2.9%, 0.7±0.7 mm and 0.2±0.3, respectively. During the one-year clinical follow-up there were no cases of death or acute MI, but two cases of target vessel revascularization (18.2%).

Conclusion : Local delivery of ^{99m}Tc-HMPAO, a novel radiotherapy, can be used safely and effectively for coronary stent restenosis.

Key Words : Coronary Artery Diseases, Restenosis, Stents, Radioisotopes

INTRODUCTION

Coronary artery disease, such as angina pectoris and myocardial infarction, has sharply increased over the last 10 years, becoming a major reason of Korean adult death. Percutaneous transluminal coronary angioplasty (PTCA) was introduced to treat coronary artery disease, but caused restenosis in approximately 30~40% of cases by negative remodeling of the injured artery and neointima formation[1], and acute vessel closure, due to intimal dissection and

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thrombus formation, in approximately 10%[2]. Coronary artery stenting lessened acute vessel complications from 10-15 to less than 1% after balloon dilation, establishing it as a universal PCI[3]. However, 20~30% stent restenosis occurs due to neointima formation, a problem that still remains to be resolved[4].

Various trials have recently been conducted on the prevention of stent restenosis following PCI, with local delivery of beta or gamma rays having proved effective[5-7]. Herein, it is hypothesized that intracellular irradiation of the media and adventitia of the porcine coronary artery, by locally delivered ^{99m}Technetium hexamethyl propylene amine oxime (^{99m}Tc-HMPAO), could inhibit neointimal hyperplasia after a stent over dilation injury.

The local delivery of ^{99m}Tc-HMPAO has been shown to have a preventive effect on neointima hyperplasia and in-stent restenosis in a porcine coronary artery stent restenosis model. Therefore, whether the local delivery of ^{99m}Tc-HMPAO has a preventive effect on restenosis and is safety on humans were assessed.

SUBJECTS AND METHODS

1. Animal experiments

Animal Preparation

Domestic pigs, 25~35 kg in weight, were premedicated with 300 mg aspirin, 180 mg diltiazem, and 500 mg ticlopidine. Induction of general anesthesia was accomplished with intramuscular injections of ketamine (12 mg/kg) and xylazine (8 mg/kg). Local anesthesia of the mid-cervical region, using 2% lidocaine, was also applied prior to exposure and cut-down of the carotid artery using a midline cervical approach. The left carotid artery was cannulated with an 8 Fr. sheath. Under fluoroscopic guidance, with a C-arm (BV-25 Gold, Phillips, Best and Heerlen, Netherlands), catheters (7-8 Fr.) were advanced to the coronary ostia. Throughout the duration of the invasive procedures, the pigs were supplied with continuous oxygenation via face masks. Saline was infused through the auricular vein. The electrocardiography and blood pressure were monitored continuously. This study was approved by the Ethical Research Committee of the Chonnam National University Hospital.

Measurement of Local Radiopharmaceutical Delivery

Twelve pigs received a local delivery of radiopharmaceuticals with either ^{99m}Tc-pertechnetate (n=5) or ^{99m}Tc-HMPAO (n=7). Upon positioning of the catheter at the target region of the coronary artery, 1,110 MBq (555 MBq/mL) of the radiopharmaceutical were infused, at a rate of 1 mL/min, followed by infusion of physiologic saline for 5 minutes. The actual radiation dose delivered was determined using a dose calibrator on dissected coronary arteries. The residual radiation in the syringe and catheters were quantified and subtracted from the initial administered dose.

Local Radiopharmaceutical Delivery in the Coronary Artery Injury & Restenosis Model

Experiments were performed on ten pigs. In the control group (n=3), balloons were attached to Palmaz-Schatz stents (Johnson & Johnson, Piscataway, NJ) and inflated in the coronary artery to 1.3 times the reference vessel diameter. In the experimental group (n=7), 1,110 MBq (555 MBq/mL) ^{99m}Tc-HMPAO was delivered via a Dispatch Catheter (Boston Scientific, Boston, MA) prior to balloon dilation, as described for the control group. ^{99m}Tc-HMPAO was infused using an infusion pump, such that a 1,110 MBq dose would occupy 2 mL of the syringe, at a rate of 1 ml/min followed by infusion of physiologic saline for 5 minutes. The diameter of the Dispatch catheter and balloon selection (3-4 mm) was determined by quantitative analysis of the coronary angiograms. Spiral balloons on the Dispatch Catheter were deployed with 4 atm of pressure. During local delivery of the ^{99m}Tc-HMPAO, a radiocontrast dye was infused through the central channel to demonstrate the blood flow distal to the Dispatch catheter. Precautions were taken to avoid ischemia in the distal segment during the ^{99m}Tc-HMPAO delivery. A Palmaz-Schatz stent was placed in the right coronary artery (RCA) and expanded with 8 atm of pressure for 20 sec using a non-compliant PTCA balloon catheter and standard indeflater. Neither heparin nor nitrates were administered after placement of the stent. Upon completion of the experiments, the carotid artery was repaired, and the incision site was sutured. Follow-up angiograms were obtained four weeks after the experiments. Quantitative analysis was performed with an image analyzer (Cardio 500, Kontron Inc., Eden Prairie, MN).

Autoradiography

One pig was sacrificed after the local ^{99m}Tc-HMPAO delivery. The hearts was extracted, rinsed, and the epicardial coronary arteries identified. A large section, including the entire coronary artery and surrounding myocardium, was obtained. Sections were placed in a container with dry ice vapor and packed in dry ice. Using a microtome, 20~40 mm sections were made, mounted on glass slides, and air-dried. In a darkroom, the glass slides were covered with X-ray film (NMB film) and stored at 70℃.

The exposure times were 2, 8 and 18 hours. After developing the autoradiographs, the glass slides were stained with Hematoxylin and Eosin (H&E) [8, 9]. Densitometry was performed using Image Pro Plus (Media Cybernetics, Silver Spring, MD).

Radiation Dosimetry

The amount of radiation absorption in the vascular wall was determined according to the Monte Carlo Simulation Study using the EGS4 code. A cylindrical model was implemented in the description of the vessel. Assuming a 1 mm central lumen, the intima, media and adventitia were described as concentric cylinders, with wall thickness of 0.27, 0.82 and 0.12 mm, respectively. The distribution of ^{99m}Tc-HMPAO was determined by setting the target volume to the smooth muscle layer. A computer analysis was repeated until the standard deviation (SD) was less than or equal to 5%.

Thierens et al.[10] reported that intracellular radiation in lymphocytes, by Auger electrons from ^{99m}Tc-HMPAO, was equivalent to 20 to 30 times the external x-ray irradiation. Thus, the theoretical dosimetry was calculated as the number of cells in the coronary arterial wall exposed to intracellular irradiation from ^{99m}Tc-HMPAO.

Histopathology & Immunocytochemistry

After follow-up coronary angiography, the pigs were sacrificed by an intravenous injection of barbiturates or KCl. The extracted porcine heart was fixed in 10% formalin solution for 24 hr. The stents in the isolated coronary arteries were easily identified under fluoroscopic guidance and were also readily palpable. Each coronary artery was dissected to remove the stented portion, including a 1 cm vessel segment both proximal and distal to the stent. Sections of the stented portion were taken at 2~3 mm intervals with a stereomicroscope to avoid distortion of or damage to the artery. Each section of the stented portion of the coronary artery was H&E stained. In order to assess neointimal proliferation, immunohistochemistry was performed using a murine monoclonal antibody (clone PC 10, Dako, Carpinteria, CA) to the proliferating cell nuclear antigen (PCNA). All morphometric analyses were performed using image analysis systems, according to previously established methods[11, 12]. The lumen diameters were determined using calibrated digital microscopic planimetry. Subtraction of the cross-sectional areas of the lumen (luminal area), internal elastic lamina (IEL) and external elastic lamina (EEL) from that of the vessel wall yielded the cross-sectional area of the neointima and media. Reference values for the vessel wall thickness were obtained from the averages of those found 1 cm proximal and distal to the stented region. The neointimal cross-sectional area was determined by subtracting the luminal area from that demarcated by the IEL. The degree of restenosis was calculated as the percent area of restenosis (%) = 100 (1-luminal area / IEL area).

Statistical analysis

All data are shown as the average ± standard error, and the comparison between the two groups was performed using unpaired Student's *t*- and Chi-squared tests. A *p* value less than 0.05 was considered as significant.

2. Clinical Study

Subjects

Of the patients attending the heart center the Chonnam National University Hospital between June and Oct. 2001, 11 (10 males, mean age 62.4±5.7 years) that had had a coronary artery stent restenosis lesion and simultaneous local delivery of ^{99m}Tc-HMPAO the same way as in the animal experiment following PCI, were entered onto this study

^{99m}Tc-HMPAO local delivery

The subject patients were administered 1.100MBq (30 mCi)/2 mL, with the Dispatch Catheter™ following a successful PCI, in the same way as in the animal experiments. Both pre- and post-PCI, electrocardiogram, CBC, and chemistry tests were performed.

Inclusion and exclusion criteria of target lesion

From quantitative coronary angiography, some patients were chosen whose vessel diameter and lesion length were 2.5-4.0 and under 20 mm, respectively. The informed consent of these patients were obtained. Patents with acute myocardial infarctionwithin 72 hours, a thrombus-containing target lesion, graft vessel PCI and chronic total occlusion were excluded, and those with more than 30% remaining stenosis post-PCI and no complications, such as coronary artery dissection, acute vessel closure or myocardial infarction, were included.

Coronary Angiography and PCI

Coronary angiography was performed before and 6 months after PCI. Significant coronary artery stenosis was defined as over 50% stenosis of the major coronary artery or of the diameter of the major branch. The morphological classification of the stenotic areawas in accordance with the guidelines of the American College of Cardiology/American

Table 1. Local delivery efficacy of radiopharmaceuticals into the porcine coronary artery

Radiopharmaceuticals	Number	Delivery rate
Tc-99m HMPAO	4	3.17% (2.68, 2.86, 4.15, 3.0)
Tc-99m Chitosan	1	1.49%
I-131	4	0.55% (0.11, 0.18, 0.61, 1.3)
Tc-99m pertechnetate	2	0.01% (0.01, 0.01)
Sr-85 Chitosan	1	0.01%

Heart Association (ACC/AHA). The reference vessel and minimal luminal diameters were measured using the Philips H5000 or Allular DCI program pre and post-PCI. Every patients took aspirin (100~200 mg) and either ticlopidine (500 mg) or clopidogrel (75 mg) one or twice a day pre-PCI, and aspirin constantly and ticlopidine or clopidogrel for 6 months post-PCI. PCI was undertaken on the coronary artery lesion using the currently preferred techniques. The femoral artery sheath was removed 6 hours post-PCI, and heparin was only administered if there was complication. Compression of the vascular access site was performed for 20 minutes by a well-trained doctor, and then pressed employing a Femostop device. Following PCI, those cases where the remaining restenosis was under 30%, myocardial infarction was not observed on EKG, cardiac enzyme assay, and major complications, such as emergent coronary artery bypass graft (CABG), and coronary revascularization, or death did not doccur, were considered successful. In-stent restenosis was classified by the criteria of Mehran et al[13], which was deemed to have occurred when the restenosis was over 40% that of the normal diameter.

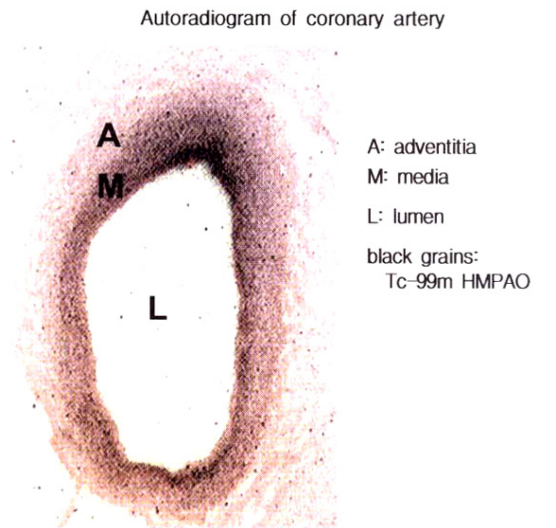

Figure 1. Autoradiography of a porcine coronary artery after an 18-hour exposure. Grains of ^{99m}Tc-HMPAO were distributed mainly in the intima and media of the coronary artery. The relative radioactivities of each layer of the vessel wall were 7.6, 59.7, 11.2 and 21.5% in the intima, media, adventitia and the surrounding myocardium, respectively.

Follow-up and MACE

As the follow-up the major adverse cardiac events (MACE) and survival were analyzed once a month at the out patients department, In-hospital MACE were defined as cardiac death, myocardial infarction, CABG, any stroke, and target lesion revascularization. The primary end point was the result of quantitative coronary angiography from the follow-up coronary angiography by 6 months; the second end point was cardiac death, myocardial infarction, target vessel revascularization or a stroke.

RESULTS

1. ^{99m}Tc-HMPAO Local Delivery Rate and Autoradiogram

The delivery of ^{99m}Tc-HMPAO into the coronary arterial wall was 3.17±0.67%, while that of ^{99m}Tc-pertechnetate was 0.01±0.01% (Table 1). Numerous small grains were shown to be distributed in coronary arterial wall after 18 hour exposure in autoradiogram. No grains appeared in the autoradiograph of the control sections. The coronary artery and surrounding myocardium had clearly appreciated. The relative radioactivities of each layer of the vessel wall were 7.6, 59.7, 11.2 and 21.5% in the intima, media, adventitia and surrounding myocardium, respectively. The radioactive particles in the vessel wall were unevenly distributed in the intima area: media area: adventitia area was 1:7:2 (Figure 1).

2. Assessment of the Amount of ^{99m}Tc-HMPAO Absorbed

The dosimetry in the smooth muscle layer of the porcine coronary arteries, as determined by the Monte Carlo Simulation Study, was 0.67±0.14 Gy (range, 0.45-0.94 Gy). Assuming the diameter of the porcine coronary artery to be 1 mm, the length of the Dispatch catheter micropores 2 cm, the thicknesses of the media and adventitia 0.94 μm and the diameter of a cell to be 10 μm, then the number of cells exposed to ^{99m}Tc-HMPAO would be about 1×10^8. The dose of ^{99m}Tc-HMPAO delivered to the porcine coronary

Table 2. Quantitative coronary angiographic findings in irradiated porcine coronary arteries (Group I) and control arteries (Group II)

	Group I		Group II	
	baseline	After 4weeks	baseline	After 4weeks
PRD (mm)	2.90±0.2	2.93±0.8	2.81±0.2	2.79±0.1
DRD (mm)	2.60±0.3	2.73±0.9	2.13±0.2	2.25±0.4
RD (mm)	2.75±0.2	2.83±0.8	2.60±0.0	2.53±0.2
DS (%)	7.28±5.5		16.43±3.7*	

PRD, proximal reference diameter; DRD, distal reference diameter; RD, reference diameter; % DS, % diameter stenosis; *, p<0.05

Table 3. Histopathological assessment of irradiated porcine coronary arteries (Group I) and control arteries (Group II)

	Group I	Group II	*p*
Neointima area (mm^2)	1.25±0.60	2.76±0.40	0.002
Media area (mm^2)	1.43±0.50	1.11±0.30	0.336
Area stenosis (%)	27.1±6.3	53.5±5.2	0.0001

arterial wall by the Dispatch Catheter in this study was 35.19±7.44 MBq, which was 3.17±0.67% of 1,110 MBq. Therefore, the exposed dose to the cells in the coronary arterial wall, according to Thierens et al[10], was equivalent to 20 to 30 Gy of external X-ray irradiation[10].

3. Animal experiment's quantitative coronary angiography and histopathological analysis

On quantitative coronary angiographic analysis, the percentage diameter of stenosis in the group receiving ^{99m}Tc-HMPAO was significantly lower than that seen in the controls (7.28±5.50% in group I and 16.43±3.70% in group II, $p<0.05$) (Table 2). On histopathological analysis, there was no difference in the media area between the two groups; 1.4±0.5 and 1.1±0.3 mm^2 in groups I and II, respectively. The neointima areas were 1.2±0.6, and 2.7±0.4 mm^2, the histopathological stenosisareas were 27.1±6.3 and 53.5±5.2% in groups I and II, respectively, and thus the group that had undergone radiotherapy had remarkably ssmaller neointima and histopathological stenosis areas than the other group (each p=0.002, p=0.001) (Table 3, Figure 2).

4. Patients' baseline characteristicson clinical study

In 10 male patients (90.0%) in this clinical study, there were 1 and 8 with stable and unstable angina pectoris, respectively, and of those with old MI there were 2, 7, 7, 3 and 1 with cardiac risk factors, hypertension, smoking, diabetes mellitus, and hypercholesterolemia. The ejection fraction was 63.2±5.8% (Table 4).

5. The Results of whole body and heart scan

On a whole body and heart SPECT (Figure 3), 27.5±5.67% of the delivery dose was observed in the heart area.

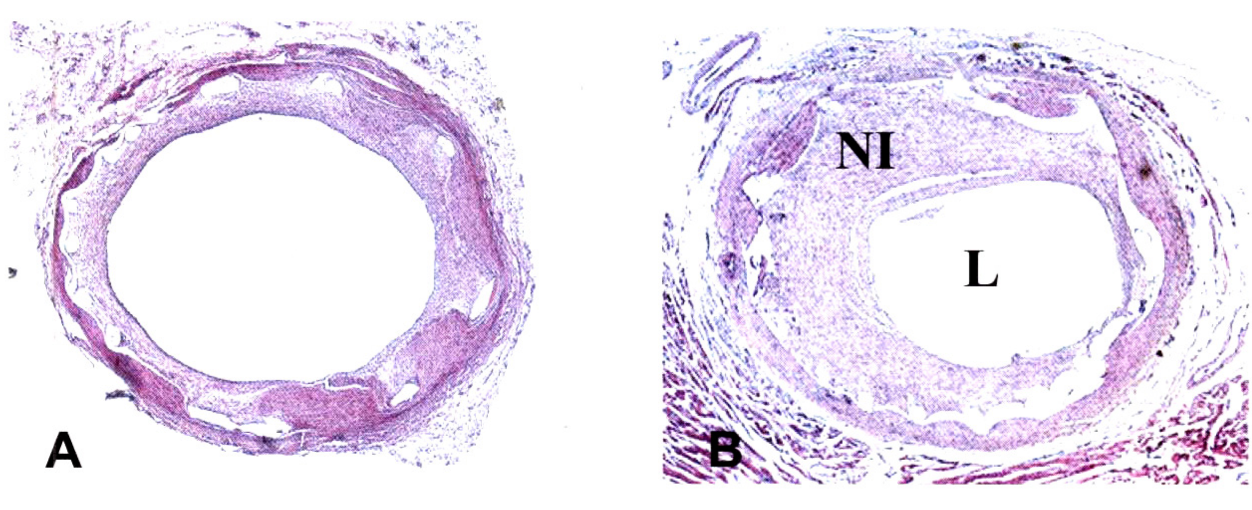

Figure 2. Histopathological findings of the local delivery of ^{99m}Tc-HMPAO (A) and the control (B) porcine coronary arteries. A higher volume of neointima and degree of area stenosis was observed in the control than the irradiated artery. NI, neointima; L, lumen.

Table 4. Baseline clinical characteristics of the patients

	Patients (n=11)
Age (year)	62.4±5.7
Male (%)	10 (90.9)
Clinical diagnosis (%)	
Stable angina pectoris	1 (9.0)
Unstable angina pectoris	8 (72.7)
Acute myocardial infarction	0 (0.0)
Old myocardial infarction	2 (18.1)
Risk factor (%)	
Hypertension	7 (63.6)
Diabetes mellitus	3 (27.3)
Hypercholesterolemia	1 (9.0)
Smoking	7 (64.2)
Family history	1 (9.0)
Ejection fraction (%)	63.2±5.8

Table 5. Coronary angiographic characteristics

	Patients
Diseased vessels (%)	
Left anterior descending artery	4 (36.4)
Right coronary artery	4 (36.4)
Left circumflex artery	3 (27.2)
ACC/AHA classification (%)	
Type B_1	3 (27.2)
Type B_2	4 (36.4)
Type C	4 (36.4)
Type of stent restenosis (%)	
Type I	3 (27.2)
Type II	6 (54.5)
Type III	1 (9.0)
Type IV	1 (9.0)
Lesion length (%)	
< 10 mm	2 (18.1)
10 ~ 15 mm	5 (45.5)
15 ~ 20 mm	4 (36.4)
Coronary intervention (%)	
Balloon angioplasty	10 (90.9)
Cutting balloon angioplasty	1 (9.0)

ACC/AHA, American College of Cardiology/American Heart Association

6. The results on coronary angiography

The locations of the lesion vessels were the left anterior descending artery, 4 the right coronary artery and 3 the left circumflex artery in 4, 4 and 3, respectively; the types of lesion were the B_1, B_2 and types, by the ACC/AHA classification, in 3, 4 and 4, respectively. Types of stent restenosis were I, II, III, IV in 3, 6, 1 and 1, respectively; with lesion lengths of restenosis under 10, and 10~20 mm in 2 and 9, respectively. A balloon angioplasty was carried out in 10 patients and a cutting balloon angioplasty in a further 1. A quantitative coronary angiographic analysis

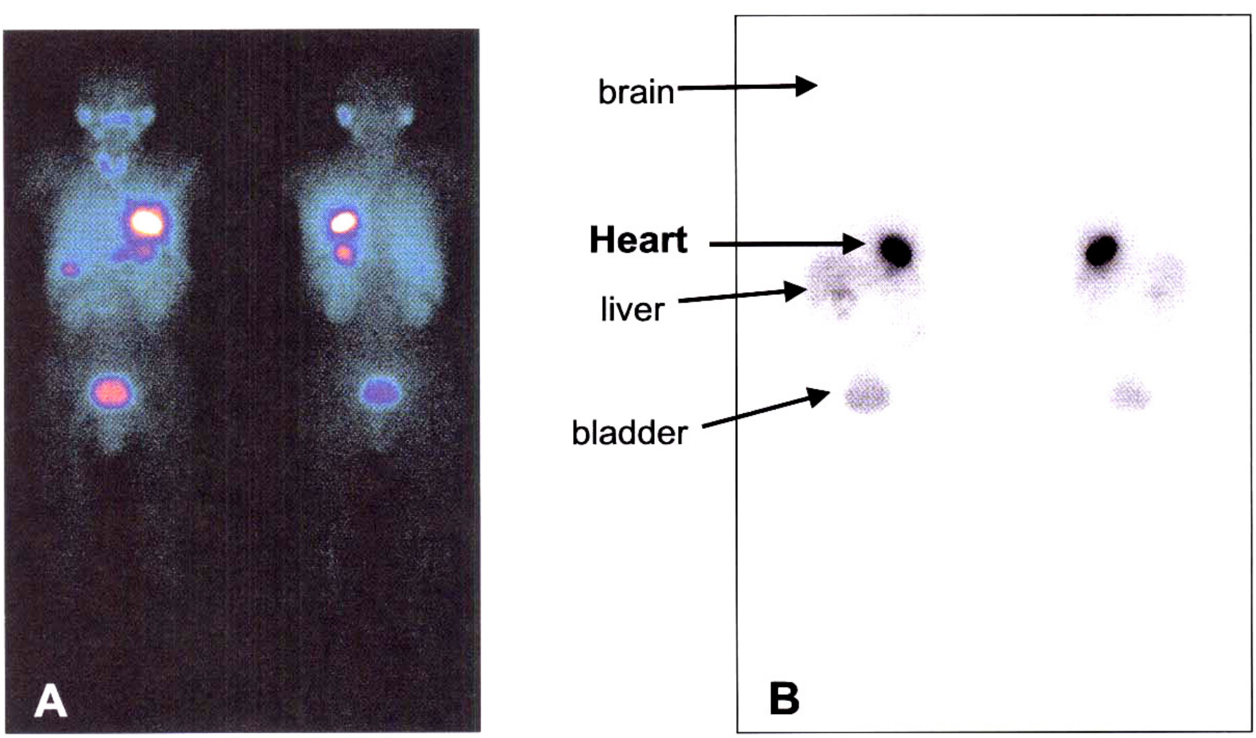

Figure 3. Whole body scan finding after the local delivery of ^{99m}Tc-HMPAO. The distribution rate was calculated as 100× (count of target organ / count of whole body), and in this case was 26.7% 3 hours after the intracoronary local delivery of ^{99m}Tc-HMPAO. A higher uptake by the heart was noted in the whole body scan.

Table 6. Quantitative coronary angiographic results

	Pre-PCI	Post-PCI	Follow-up
Proximal reference diameter (mm)	2.99±0.20	3.08±0.33	2.99±0.34
Distal reference diameter (mm)	2.77±0.19	2.75±0.21	2.80±0.38
Reference diameter (mm)	2.87±0.21	2.93±0.25	2.88±0.39
Minimal luminal diameter (mm)	0.40±0.31	2.98±0.29	2.08±0.83
Diameter stenosis (%)	84.2±9.52	16.3±11.0	27.7±9.1
Lesion length (mm)	13.8±8.9	-	-
Acute luminal gain (mm)	-	2.57±0.38	-
Late lumen loss (mm)	-	-	0.79±0.78
Loss index	-	-	0.23±0.30

PCI, percutaneous coronary intervention

showed better results compared to pre-PCI to post-PCI that minimal luminal diameter was from 0.04±0.31 mm to 2.89±0.29 mm, diameter stenosis rate was from 84.20±9.52% to 16.35±11.06% and acute luminal gain was 2.57±0.38. On the follow-up coronary angiography, the minimal luminal diameter, diameter stenosis rate, lumen loss and loss index were 2.08±0.83 mm, 27.7±9.1%, 0.79±0.78 mm and 0.23±0.30, respectively (Table 5, 6).

7. In-hospital MACE and 1 year Follow-up Results

There were no major in-hospital cardiac complications. All the patients were followed up for 1 year. A follow-up coronary angiography was performed on 9 patients (81.8%) and 2 of them had restenosis (22.2%). During the 1 year clinical follow-up, there wereas no cardiac deaths, acute myocardial infarctions, strokes or CABG, but 2 (18.2%) target vessel revascularizations (Table 7).

8. Side-effects and Lab. findings

No patients had neither side-effects nor complications related to the PCI, and all the results from laboratory examinations performed pre and post-PCI and in after 6th month were within normal ranges, with no considerable differences (Table 8).

Table 7. One-year clinical follow-up

	Patients
Success rate (%)	11 (100)
Follow-up coronary angiogram (%)	9/11 (81.8)
Restenosis	2/9 (22.2)
Composite clinical end point (%)	
Cardiac death	0 (0.0)
Acute myocardial infarction	0 (0.0)
Target lesion revascularization	2 (18.2)
Bypass surgery	0 (0.0)

DISCUSSION

Stent restenosis due to neointima hyperplasia is considered a major difficulty of PCI. The exact reason for the neointima hyperplasia was not clear, but was known to be related to the proliferation of the drelocated smooth muscle cells from the vessel media layers. Various treatments; gene therapy, radiotherapy, drug-eluting or coated stents, are being trialed for the prevention of neointima hyperplasia, with local

Table 8. Laboratory findings before, and follow-up after, percutaneous coronary intervention

	Baseline	Follow-up
WBC (/μL)	7.2±1.5	6.3±1.7
Hemoglobin (g/dL)	14.2±1.4	14.2±0.6
Platelet (K/μL)	196.5±44.5	213.5±71.1
AST (IU/L)	25.0±14.7	19.5±5.8
ALT (IU/L)	34.2±15.0	28.2±5.6
BUN (mg/dL)	13.7±2.1	15.5±3.4
Creatinine (mg/dL)	0.96±0.17	0.95±0.7
Cholesterol(mg/dL)	172.0±23.4	172.8±22.2
Na^+ (mg/dL)	141.1±1.3	137.7±4.4
K^+ (mg/dL)	4.3±0.2	4.3±0.9

WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen radiation delivery to the coronary artery being study as one of the effective treatments[5-7, 14].

Therefore local radiation delivery could prevent proliferation of the neointima. Beta and gamma rays, as radioisotopes for local radiation delivery, are being studied. Gamma-rays had good results in a study of ^{192}Ir-treated patients with stent restenosis[15]. Beta-rays were used, by Verin et al, for the study of local radiation delivery[16]. which showed the 1st indication of radiotherapy in the coronary artery iwas an in-stent restenosis lesion.

^{166}Holmium(^{166}Ho-coated balloon) was developed for us, with the support of the Korea Atomic Energy Research Institute, for in-stent restenosis lesions which improved the function of the coronary artery endothelial cells and suppressing neointima formation within the stent[17, 18] in a porcine coronary artery stent restenosis model. The local delivery of ^{99m}Tc-HMPAO has also been reported to have an effect in preventing stent restenosis and controlling in-stent neointima hyperplasia in a porcine coronary stent restenosis model[19].

^{99m}Tc-HMPAO is a lipophilic agent that can pass through the blood-brain barrier as well as diffuse through cell membranes. Once internalized in the intracellular compartment, ^{99m}Tc-HMPAO is converted to a water-soluble form, which for the most part becomes stored in cells. An understanding of theis nature of ^{99m}Tc-HMPAO has led to the development of applications in the imaging of cerebral blood flow and the detection of infection by the labeling of leukocytes[20-22]. From the labeling of heparin with ^{99m}Tc, Camenzind et al.[23] reported that 2.5±2.4% of the heparin could be locally delivered into the human coronary arterial wall by a Dispatch Catheter. The present study revealed that 2~5% (mean 3.17%) of the heparin can be delivered locally, which was similar to the results of Camenzind et al[23]. The delivered ^{99m}Tc-HMPAO was believed to be localized inside the arterial cells due to the lipophilicity of ^{99m}Tc-HMPAO.

Little data is available concerning the irradiation dosimetry of intracellular ^{99m}Tc-HMPAO, other than for lymphocytes. Accurate calculation of the dose is very difficult because of the different Auger electron groups emitted in the decay of ^{99m}Tc, the distribution of the radioactivity within the cells and the limits of the classical dosimetry methods. Thierens et al.[10] reported that the radiation damage in 1.3×10^8 lymphocytes, due to self-irradiation with 740 MBq of ^{99m}Tc-HMPAO, was estimated to be equivalent to that caused by 26 Gy of X-rays. They estimated the dose using a biological dosimetry method of a micronucleus assay, with extrapolation of the data. In their experiment the total radioactivity in 10^7 lymphocytes was 28 MBq after a 740 MBq dose, and this dose almost completely inhibited the ability of the lymphocytes to proliferate. A few other studies have reiterated that ^{99m}Tc-HMPAO could deliver doses of radiation equivalent to those provided by high dose external radiation[24-26]. The calculated absorbed dose to the cells in a labeling procedure for 10^8 granulocytes with 500 MBq ^{99m}Tc, with a bisalt method without pretinning, yielded a value of 17.7 Gy, assuming an uniform distribution of intracellular activity[24].

Herein, about 1×10^8 cells were assumed to be irradiated from 37 MBq of ^{99m}Tc-HMPAO, which was located intracellularly. According to the report of Thierens et al., the exposure dose from intracellular ^{99m}Tc-HMPAO was equivalent to 20 to 30 Gy of external X-ray irradiation[10]. In suppressing the proliferation of damaged vessel walls, 8-30 Gy was found to be effective[27, 28]. So the dose of intracellular irradiation from ^{99m}Tc-HMPAO seems to be adequate to inhibit the proliferation of medial and adventitial cells. Autoradiography confirms that the ^{99m}Tc-HMPAO is mainly retained in the media and adventitia of coronary arterial wall. The calculation for the dosimetry in the coronary arterial wall, according to the Monte Carlo simulation study, showed only 0.67±0.14 Gy, which was not relevant to the result of this study, where the neointimal proliferation was significantly inhibited. The rate of ^{99m}Tc-HMPAO absorption was estimated from the injected amount vs. amount found in the heart on the whole body and heart SPECT of the patients. On the SPECT, 27.5±5.67% of injected amount wasobserved in the heart cells.

The best merit of ^{99m}Tc-HMPAO on local delivery is its ease of application. That is to say, even in hospital with no atomic facility, it would be possible to produce material in the form of a commercial kit within 1 hour, which would be capable of being used for radiotherapy, as long as there is a doctor able to control the radioisotopes. It is also economical, as it costs less than other radiotherapies.

In this study, there were no MACE, with about 20% target vessel revascularization, compared to the 16~31% in GAMMA I[6], START[29] and INHIBIT trials[30], and gave a good result. Even if though this was a small trial, the patients' lesions were relatively longer than 10mm, and considering 8 of our patients that did not have focal, but diffuse in-stent restenosis lesions, our result can be regarded as good. All patients in this study had no conventional complications of radiotherapy, such as late stent thrombosis or edge failure, which was probably due to the few patients.

The first limitation of this study was that, there were only a few patients. However, an advanced study will be required as a trial of this novel radiotherapy. The second limitation was the accuracy of the dosimetry of irradiation to the tissue may have been lower than that estimated. The determination of the radiation doses, according to the amount absorbed by the whole body and heart, may cause low accuracy as the delivery catheter thickness, lesion vessel size and irradiating time were not taken into consideration. The third limitation related to the Dispatch Catheter^{TM} used for the local delivery, which could be a cause for practical concern.

In conclusion, the local administration of 1,110 MBq ^{99m}Tc-HMPAO into the coronary arterial wall, using a Dispatch Catheter, delivered 35.1±7.44 MBq, which was 3.1 ±0.67% of the injected dose, mainly into the media and adventitia of the coronary arterial wall. This novel local radiotherapy with ^{99m}Tc-HMPAO is feasible to cure in-stent restenosis lesions in animals and clinical experiments. However, further clinical studies might be required in the future.

REFERENCES

1) Bittl JA. *Advances in coronary angioplasty. N Engl J Med 335: 1290-1302, 1996*
2) Lincoff AM, Popma JJ, Ellis SG, Hacker JA, Topol EJ. *Abrupt vessel closure complicating coronary angioplasty: clinical angiographic and therapeutic profile. J Am Coll Cardiol 19:926-935, 1992*
3) Serruys PW, de Jaegere P, Kiemeneij F, Macaya C, Rutsch W, Heyndrickx G, Emanuelsson H, Marco J, Legrand V, Materne P. *A comparison of balloon-expandable stent implantation with balloon angioplasty in patients with coronary heart disease. N Eng J Med 331:489-495, 1994*
4) Fischman DL, Leon MB, Baim DS, Schatz RA, Savage MP, Penn I, Detre K, Veltri L, Ricci D, Nobuyoshi M. *A randomized comparison of coronary stent placement and balloon angioplasty in the treatment of coronary artery disease. N Engl J Med 331:496-501, 1994*
5) Waksman R, White RL, Chan RC, Bass BG, Geirlach L, Mintz GS, Satler LF, Mehran R, Serruys PW, Lansky AJ, Fitzgerald P, Bhargava B, Kent KM, Pichard AD, Leon MB. *Intracoronary gamma-radiation therapy after angioplasty inhibits recurrence in patients with in-stent restenosis. Circulation 101:2165-2171, 2000*
6) Leon MB, Teirstein PS, Moses JW, Tripuraneni P, Lansky AJ, Jani S, Wong SC, Fish D, Ellis S, Holmes DR, Kerieakes D, Kuntz RE. *Localized intracoronary gamma-radiation therapy to inhibit the recurrence of restenosis after stenting. N Engl J Med 344:250-256, 2001*
7) Waksman R, Bhargava B, White L, Chan RC, Mehran R, Lansky AJ, Mintz GS, Satler LF, Pichard AD, Leon MB, Kent KK. *Intracoronary beta-radiation therapy inhibits recurrence of in-stent restenosis. Circulation 101:1895-1898, 2000*
8) Tomoike H, Ogata I, Maruoka Y, Sakai K, Kurozumi T, Nakamura M. *Differential registration of two types of radionuclides on macroautoradiograms for studying coronary circulation: concise communication. J Nucl Med 24:693-699, 1983*
9) Greiff J. *Bone healing in rabbits after compression osteosynthesis, studied by Tc-99m(Sn)polyphosphate scintimetry and autoradiography. J Nucl Med 22:693-698, 1981*
10) Thierens HM, Vral AM, van Haelst JP, van de Wiele C, Schelstraete KH, de Ridder LI. *Lymphocyte labeling with technetium-99m-HMPAO: a radiotoxicity study using the micronucleus assay. J Nucl Med 33:1167-1174, 1992*
11) Schneider JE, Berk BC, Gravanins MB, Santoian EC, Cipolla GD, Tarazona N, Lassegue B, King SB 3rd. *Probucol decreases neointimal formation in a swine model of coronary balloon injury. Circulation 88:628-637, 1993*
12) Ahn YK, Jeong MH, Kim JW, Kim SH, Cho JH, Park CS, Jung SW, Cho JG, Park JC, Kang JC. *The preventive effects of heparin-coated stent on restenosis in the porcine model. Catheter Cardiovasc Interv 48:324-330, 1999*
13) Mehran R, Dangas G, Abizaid AS, Mintz GS, Lansky AJ, Satler LF, Pichard AD, Kent KM, Stone GW, Leon MB. *Angiographic patterns of in-stent restenosis: classification and implications for long-term outcome. Circulation 100:1872-1878, 1999*
14) Jeong MH, Ahn YK, Cho JG, Park JC, Kang JC. *Successful coronary stent implantation using local NO donor delivery. J Interv Cardiol 13:191-195, 2000*
15) Teirstein PS, Massullo V, Jani S, Russo RJ, Cloutier DA, Schatz RA, Guarneri EM, Steuterman S, Sirkin K, Norman S, Tripuraneni P. *Two-year follow-up after catheter-based radiotherapy to inhibit coronary restenosis. Circulation 99:192-194, 1999*
16) Verin V, Popowski Y, de Bruyne B, Baumgart D, Sauerwein W, Lins M, Kovacs G, Thomas M, Calman F, Disco C, Serruys PW, Wijns W. *Endoluminal beta-radiation therapy for the prevention of coronary restenosis after balloon angioplasty. N Engl J Med 344:243-249, 2001*
17) Rhew JY, Jeong MH, Lee SR, Hong YJ, Lee SH, Park OY, Jeong WK, Kim W, Kim JH, Yum JH, Song HC, Bom HS, Park KB, Ahn YK, Cho JG, Park JC, Baik YH, Kang JC. *The effects of radiation using Ho-166 on endothelial function in a porcine coronary model. Korean Circ J 32:118-124, 2002*
18) Kim W, Jeong MH, Park OY, Rhew JY, Bom HS, Choi SJ, Park KB, Kim EH, Kim JH, Ahn YK, Park JT, Cho JG, Park JC, Kang JC. *Effects of beta-radiation using holmium-166 coated balloon on neointimal hyperplasia in a porcine coronary stent restenosis model. Circ J 67:625-629, 2003*
19) Jeong HJ, Bom HS, Song HC, Ahn YK, Kim NH, Cho JH, Kim EH, Jeong MH, Kang JC. *Inhibition of coronary stent restenosis by local delivery of Tc-99m HMPAO. J Nucl Med 41:797S, 2000*
20) Huang WT, Lo JM, Kao CH, Wang SJ. *^{99m}Tc phenylene imine phenol as a potential leukocyte labeling agent. Nucl Med Commun 18:66-69, 1997*
21) Asenbaum S, Brucke T, Pirker W, Pietrzyk U, Podreka I. *Imaging of cerebral blood flow with technetium-99m-HMPAO and technetium-99m-ECD: a comparison. J Nucl Med 39:613-618, 1998*
22) Borch K, Greisen G. *^{99m}Tc-HMPAO as a tracer of cerebral blood flow in newborn infants. J Cereb Blood Flow Metab 17:448-454, 1997*
23) Camenzind E, Bakker WH, Reijs A, van Geijlswijk IM, Boersma E, Kutyk MJ, Krenning EP, Roelandt JR, Serruys PW. *Site-specific intracoronary heparin delivery in humans after balloon angioplasty: a radioisotopic assessment of regional pharmacokinetics. Circulation*

96:154-165, 1997

24) Skretting A, Benestad HB, Sundrehagen E. *Whole body distribution of ^{99m}Tc labelled autologous human granulocytes and radiation dose to cells and organs. Eur J Nucl Med 14:1-7, 1988*
25) Makrigiorgos GM, Adelstein SJ, Kassis AI. *Limitations of conventional internal dosimetry at the cellular level. J Nucl Med 30:1856-1864, 1989*
26) Merz T, Tatum J, Hirsch J. *Technetium-99m-labeled lymphocytes: a radiotoxicity study. J Nucl Med 27:105-110, 1986*
27) Teristein P. *β-radiation to reduce restenosis: too little, too soon? Circulation 95:1095-1097, 1997*
28) Waksman R, Rodriguez JC, Robinson KA, Cipolla GD, Crocker IR, Scott NA, King SB 3rd, Wilcox JN. *Effect of intravascular irradiation on cell proliferation, apoptosis, and vascular remodeling after balloon overstretch injury of porcine coronary arteries. Circulation 96:1944-1952, 1997*
29) Popma JJ, Suntharalingam M, Lansky AJ, Heuser RR, Speiser B, Teirstein PS, Massullo V, Bass T, Henderson R, Silber S, von Rottkay P, Bonan R, Ho KK, Osattin•A, Kuntz RE. *A randamised trial of 90strontium/90yttrium beta radiation versus placebo control for the treatment of in-stent restenosis. Circulation 106:1090-1096, 2002*
30) Walksman R, Raizner AE, Yeung AC, Lansky AJ, Vandertie L. *Use of localised intracoronary β-radiation in treatment of in-stent restenosis. Lancet 359:551-557, 2002*