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Phase I clinical trial on intracoronary administration of Ad-hHGF treating severe coronary artery disease

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Abstract *Objective* Therapeutic angiogenesis is a new strategy for treatment of vascular insufficiency. Hepatocyte growth factor (HGF)-induced angiogenesis has been applied to induce the neovascularization of ischemic adult tissues in preclinical studies. This report summarizes a phase I clinical trial on the safety of adenovirus-mediated human HGF (Ad-HGF) gene transfer to treat clinically significant coronary artery disease. *Methods* The 18 patients with severe and diffused triple vessel disease determined by coronary angiography, 1–3 of the main coronary arteries not amenable to bypassing grafting and to catheter-based revascularization were assigned to 3 study groups according to the dose of Ad-HGF (from low to high), and the total dose as follows: 5×10^9 pfu (group A, $n = 6$); 1×10^{10} pfu (group B, $n = 6$); 2×10^{10} pfu (group C, $n = 6$). Arterial gene transfer was performed by over-the wire balloon to the distal of the accessible artery or otherwise the ostium of the target vessels by diagnostic coronary catheter. General safety parameters and cardiac-specific parameters were measured through the preoperative period and on day 7, 21, and 35 postoperatively. The safety and tolerance of Ad-HGF for patients were evaluated according to functional and cytological assessments. *Results* During the acute phase up to day 35 and at 11–14 months of follow-up there were no serious adverse events. A mild fever during the first 3 days was not present at day 4, and no long term or paroxysmal fever was found. There were no acute alterations in hemodynamic parameters and the electrocardiogram remained normal. No serious pericardial effusion was reported and there were no arrhythmia on Holter registrations. Moreover, the serum levels of HGF were not changed and the serum anti-adenovirus in the patients was not detected up to day 35. *Conclusions* The present study demonstrates that it is feasible to safely use an adenovirus gene-transfer vector to deliver the human hepatocyte growth factor gene to individuals with clinically significant coronary artery disease by direct intracoronary injection. However, a great deal of additional work must be done before administration of Ad-HGF can be recommended for clinical practice.

Keywords Angiogenesis · Gene therapy · Genetics · Coronary disease

Introduction

A new experimental strategy for treating myocardial ischemia is to induce neovascularization of the heart by the use of "angiogens" mediators inducing the formation of blood vessels [1, 2]. This approach is based on the current knowledge of the adult heart that the genes coding for angiogens and their receptors are expressed at low levels, apparently insufficient in most individuals to provide robust formation of collateral circulation in response to chronic ischemia [3, 4]. Hepatocyte growth factor (HGF) is a heterodimeric pluripotent growth factor with an 80-kDa apparent molecular weight which has been shown to have

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potent angiogenic actions on various types of cells [5]. It has been shown to be induced in skeletal muscle after ischemic injury [6] and is implicated in capillary endothelial cell (EC) regeneration in the ischemically injured myocardium [7]. In addition, HGF has anti-apoptotic effects [8], blocking the programmed cell death that is known to contribute significantly to the development of ischemic heart failure [9]. Due to the potential effects on angiogenic, anti-apoptotic, anti-fibrotic, and anti-inflammatory benefits, HGF has attracted increasing attention in the studies of ischemic heart diseases lately [10–13]. HGF gene transfer has been shown to be effective to result in angiogenesis in several ischemic animal models [14, 15].

We have previously constructed a replication-deficient adenovirus carrying HGF gene (Ad-HGF) previously. In experimental animal models the adenovirus can mediate high expression of human HGF leading to increase the number of functional arterioles and improve collateral artery growth [16]. Preclinical evaluations showed that Ad-HGF is effective in both acute and chronic myocardial ischemia models and no apparent toxicity and mutation effects were observed in rat, minipig and rhesus. Its clinical application has been approved in China. The aim of this phase I clinical study is to evaluate the safety of Ad-HGF on individuals with clinically significant coronary artery disease.

Materials and methods

Materials

Ad-HGF is constructed and produced by Chinese Academy of Military Medical Sciences [17]. The specifications of Ad-HGF product include: endotoxin is under 10 EU/ml, bovine serum albumin is under 50 ng/ml, rudimental DNA from 293 cells is under 2 ng/ml and the rudimental protein is under 20 ng/ml. The viral concentration is higher than 1.0×10^{10}/ml in particles and than 1.0×10^{9}/ml in pfu.

Study design

This study was performed in the Cardiological Center of the First Affiliated Hospital to Nanjing Medical University. The trial was approved by the State Food and Drug Administration of China (ref. 2005L01181) and the Ethical Committee of the First Hospital Affiliated to Nanjing Medical University. The written consent was obtained from all patients. The patients were treated only once and were assigned to 3 study groups according to the dose of Ad(5)-HGF (from low to high); and the total dose as follows: 5×10^{9} pfu (group A, $n = 6$); 1×10^{10} pfu (group B, $n = 6$); 2×10^{10} pfu (group C, $n = 6$). Inclusion criteria for catheter-based intracoronary gene transfer were aged 50–80 years (no pregnant women), stable Canadian Cardiovascular Society class II to III angina. All 18 patients suffered from severe and diffused triple vessel disease determined by coronary angiography, 1–3 of the main coronary arteries not amenable to bypassing grafting and to catheter-based revascularization, no previous PTCA or stenting of the target vessel, and no contraindications to acetosalicylic acid or heparin. 24-h Holter monitoring was used to exclude the individuals with life-threatening arrhythmias. All subjects received the treatment of medication including aspirin, nitrates, ACE inhibitors or angiotensin II receptor blockers, beta blockers, and statins. Subjects were excluded if they had acute myocardial infarction, unstable angina, acute upper respiratory tract infection, diabetes, malignancy, kidney failure, and hepatic failure.

Gene transfer

Coronary angiography was performed via the femoral artery according to standard clinical procedures. Arterial gene transfer was performed by over-the wire balloon to distal of the accessible artery or otherwise the ostium of the target vessels by diagnostic coronary catheter. Ad(5)-HGF was diluted into 2 ml syringe with normal saline, injected within 30 s, and then flushed with normal saline. Serial coronary angiograms were obtained at the end of the infusion.

General safety parameters

The general safety parameters including body temperature, heart rate, respiratory rate, and blood pressure plus the blood parameters including aspartate aminotransferase, alanine aminotransferase, bilirubin (total, direct, and indirect), alkaline phosphatase, glucose, white blood cell count, hemoglobin, hematocrit, platelet count, electrolytes, creatinine, and serum urea nitrogen, all were measured through the preoperative period and at day 7, 21, and 35 postoperatively.

Cardiac-specific parameters

The degree of angina (on a scale of 1–4) was assessed preoperatively and at day 7, 21, and 35 postoperatively by using of a questionnaire describing set by Canadian Cardiovascular Society Classification [18]. Serial ECG was used to assess myocardial ischemia, infarction, or arrhythmia.

Enzyme-linked immunosorbent assay for HGF

The plasma level of HGF was determined by ELISA. ELISA was carried out using a mouse monoclonal antibody against human HGF (R&D Systems), horseradish peroxidase-labeled goat anti-mouse immunoglobulin, and a

spectrophotometric *o*-phenylenediamine color-developing system. The OD value was measured by Microplate Manager 450 at 490 nm wavelength.

Titer of plasma neutralizing antibody

Neutralizing antibodies against type 5 adenovirus were determined by using a cell-based plaque assay. Plasma samples from patients and control plasma were diluted (1:10–1:2,560) and incubated with 5×10^4 pfu of adenovirus carrying GFP gene (ad-GFP) at 37°C for 30 min. Then the plasma-Ad-GFP mix was applied to cultured HEK293 cells for cell-based plaque assay.

Biomarker for the tumour

The biomarker for the tumour including alphafetoprotein (AFP) (ng/ml) and carcino-embryonic antigen (CEA) (ng/ml) were monitored before treatment and at day 35 after treatment.

Patient follow-up and Assessment

All of the 18 patients were followed up for about 11–14 months by blood analysis after the injection of the adenovirus5-mediated human hepatocyte growth factor.

Data analysis

Data are presented as mean ± sd. Analysis system software SPSS 11.0 was used for all the analyses. Non-continuous demographic parameters were compared using Fisher's Accuracy Test. A level of $P < 0.05$ was considered as statistically different. Differences of baseline and postoperative characteristics of the subjects in the study groups were compared by one-way ANOVA.

Results

Patient demogram

Eighteen patients were enrolled in this study. Characteristics of the patients are shown in Table 1. There were no significant differences among the study groups.

General safety parameters

Blood routine and biochemical assays, examinations of cardiac and hepatonephric functions of all three group patients were examined before and at day 7, 21, or 35 after administration. There was no evidence of a dose-related trend towards abnormalities in any blood parameters and no differences in any blood parameters among the three groups at day 7, 21, or 35, compared with those before therapy (Tables 2–4). No evidence indicates associations of either acute/sustained hypotension or hemodynamic involvement with Ad-HGF therapy.

Cardiac-related parameters

In either group, during hospitalization and at day 7, 21, and 35 daily ECG showed no new ST changes or Q waves. In either group, 24-h Holter monitoring performed before therapy and on day 7 demonstrated no average increase in supraventricular or ventricular arrhythmias after therapy. Serial echocardiographic studies in the three groups had not provided any evidence of significant pericardial effusions.

Serum HGF level and anti-adenovirus antibodies

We detected the serum HGF levels by ELISA and plasma neutralizing antibody against adenovirus. The serum level of HGF was measured before and at day 7 after gene

Table 1 Demogram and preoperative characteristics of the study population

Parameter	Group			F or χ^2	*P*
	A	B	C		
N	6	6	6	–	–
Age, years	70.00 ± 10.60	62.50 ± 10.02	64.67 ± 7.20	1.014	0.386
Male/female, n	5/1	5/1	5/1	0.000	1.000
Temperature, °C	36.80 ± 0.33	36.55 ± 0.12	36.78 ± 0.24	1.904	0.183
Heart rate, /min	69.00 ± 8.67	74.00 ± 5.06	73.00 ± 9.72	0.645	0.538
Respiratory rate, /min	17.00 ± 1.67	17.17 ± 1.83	16.50 ± 1.76	0.234	0.794
Mean blood pressure/mmHg	96.67 ± 8.09	92.67 ± 7.10	95.00 ± 7.38	0.426	0.661
Left ventricular diastolic diameter, mm	60.50 ± 9.89	49.00 ± 9.79	53.50 ± 8.83	2.224	0.143
Left ventricular ejection fraction, %	50.92 ± 10.28	60.35 ± 13.75	55.83 ± 6.62	1.184	0.333

Table 2 Baseline and postoperative characteristics of study group A

Parameter	Baseline	Day 7	Day 21	Day 35	*P* value
T, °C	36.8 ± 0.3	36.5 ± 0.2	36.7 ± 0.2	36.9 ± 0.3	0.618
HR, /min	69.0 ± 8.7	71.3 ± 7.3	72.2 ± 9.1	72.0 ± 6.4	0.755
RR, /min	17.0 ± 1.7	16.6 ± 1.6	16.2 ± 0.4	16.3 ± 1.4	0.501
SBP, mmHg	123.3 ± 9.8	124.2 ± 8.9	120.8 ± 6.6	121.7 ± 10.8	0.894
DBP, mmHg	83.3 ± 7.5	80.3 ± 7.3	78.3 ± 5.2	82.5 ± 7.6	0.421
HB, g/l	128.3 ± 10.3	129.2 ± 5.9	130.0 ± 10.2	131.8 ± 9.1	0.919
RBC, 10^{12}/l	4.68 ± 0.77	4.68 ± 0.59	4.87 ± 0.77	5.07 ± 0.53	0.718
WBC, 10^9/l	6.78 ± 1.42	7.12 ± 1.09	6.93 ± 1.24	6.88 ± 0.91	0.968
PLT, 10^9/l	216.8 ± 36.2	216.3 ± 34.0	214.2 ± 37.4	212.8 ± 35.6	0.997
N, 10^9/l	3.10 ± 0.67	3.39 ± 0.62	3.43 ± 1.08	3.14 ± 0.64	0.926
TBIL, μmol/l	15.4 ± 3.2	15.0 ± 2.7	15.5 ± 2.7	14.9 ± 2.5	0.982
DBIL, μmol/l	5.7 ± 1.1	5.6 ± 0.9	5.8 ± 1.0	5.6 ± 1.2	0.981
GPT, U/l	22.5 ± 5.5	21.9 ± 5.9	22.2 ± 6.3	21.2 ± 5.6	0.984
GOT, U/l	19.4 ± 5.7	18.8 ± 5.7	18.9 ± 6.3	18.2 ± 6.0	0.989
LDH, U/l	182.5 ± 46.2	183.3 ± 46.5	190.0 ± 46.0	194.2 ± 37.3	0.962
BUN, mmol/l	5.4 ± 0.8	5.3 ± 0.6	5.4 ± 0.6	5.3 ± 0.6	0.998
CR, μmol/l	85.9 ± 18.9	85.8 ± 18.9	84.4 ± 17.2	85.6 ± 19.0	0.999
GLU, mmol/l	4.8 ± 0.4	4.4 ± 0.4	4.7 ± 0.4	4.3 ± 0.4	0.185

T indicates temperature; HR, heart rate; RR, respiratory rate, SBP, systolic blood pressure; DBP, diastolic blood pressure; HB, hemoglobin; RBC, erythrocyte; PLT, platelet; N, neutrophil; TBIL, total bilirubin; DBIL, direct bilirubin; GPT, glutamate-pyruvate transaminase; GOT, glutamic-oxal(o)acetic transaminase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; CR, creatinine; GLU, glucose

Table 3 Baseline and postoperative characteristics of study group B

Parameter	Baseline	Day 7	Day 21	Day 35	*P* value
T, °C	36.6 ± 0.1	36.6 ± 0.2	36.8 ± 0.2	36.8 ± 0.2	0.130
HR, /min	74.0 ± 5.1	75.3 ± 5.2	76.8 ± 4.2	73.8 ± 4.7	0.472
RR, /min	17.2 ± 1.8	17.3 ± 1.6	16.2 ± 1.7	16.2 ± 1.5	0.509
SBP, mmHg	124.7 ± 10.8	125.3 ± 8.9	124.2 ± 7.4	123.3 ± 8.2	0.966
DBP, mmHg	76.7 ± 6.1	75.5 ± 6.2	73.3 ± 4.1	70.8 ± 7.4	0.269
HB, g/l	126.8 ± 7.3	126.8 ± 5.9	126.7 ± 4.9	124.0 ± 5.1	0.805
RBC, 10^{12}/l	4.43 ± 0.49	4.36 ± 0.60	4.50 ± 0.63	4.44 ± 0.71	0.984
WBC, 10^9/l	6.30 ± 1.37	6.33 ± 1.47	6.35 ± 1.09	6.30 ± 1.23	0.980
PLT, 10^9/l	205.7 ± 39.9	215.3 ± 41.8	215.2 ± 39.2	209.0 ± 34.7	0.965
N, 10^9/l	3.20 ± 1.01	3.21 ± 0.99	3.24 ± 0.77	3.09 ± 0.79	0.992
TBIL, μmol/l	15.4 ± 3.2	11.1 ± 4.2	11.3 ± 4.6	11.1 ± 4.6	0.999
DBIL, μmol/l	4.2 ± 1.1	4.1 ± 0.9	4.3 ± 0.9	4.4 ± 1.2	0.962
GPT, U/l	26.5 ± 17.2	26.1 ± 17.3	25.4 ± 13.3	23.9 ± 12.1	0.992
GOT, U/l	19.8 ± 6.9	19.8 ± 6.8	19.9 ± 5.6	19.8 ± 6.3	0.890
LDH, U/l	198.8 ± 33.0	202.8 ± 31.8	201.0 ± 31.2	205.2 ± 29.6	0.987
BUN, mmol/l	6.2 ± 1.2	6.0 ± 1.3	5.9 ± 0.9	5.9 ± 0.7	0.982
CR, μmol/l	97.8 ± 38.2	97.1 ± 38.7	91.6 ± 26.7	92.0 ± 27.9	0.981
GLU, mmol/l	5.3 ± 1.8	5.3 ± 1.9	5.2 ± 1.7	5.1 ± 1.8	0.996

T indicates temperature; HR, heart rate; RR, respiratory rate, SBP, systolic blood pressure; DBP, diastolic blood pressure; HB, hemoglobin; RBC, erythrocyte; PLT, platelet; N, neutrophil; TBIL, total bilirubin; DBIL, direct bilirubin; GPT, glutamate-pyruvate transaminase; GOT, glutamic-oxal(o)acetic transaminase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; CR, creatinine; GLU, glucose

Table 4 Baseline and postoperative characteristics of the study group C

Parameter	Baseline	Day 7	Day 21	Day 35	*P* value
T, °C	36.8 ± 0.2	36.7 ± 0.3	36.8 ± 0.2	36.6 ± 0.3	0.425
HR, /min	73.0 ± 9.7	75.5 ± 7.1	77.0 ± 4.7	73.7 ± 8.0	0.642
RR, /min	16.5 ± 1.7	16.6 ± 1.9	16.8 ± 1.7	16.2 ± 1.9	0.818
SBP, mmHg	121.7 ± 10.8	123.2 ± 9.9	121.7 ± 4.1	122.2 ± 9.1	0.964
DBP, mmHg	81.7 ± 6.1	80.3 ± 10.3	76.7 ± 11.3	78.1 ± 8.8	0.565
HB, g/l	132.8 ± 5.6	132.7 ± 5.9	132.8 ± 7.8	131.7 ± 7.6	0.988
RBC, 10^{12}/l	4.10 ± 0.38	4.13 ± 0.51	4.10 ± 0.52	4.08 ± 0.47	0.998
WBC, 10^{9}/l	6.70 ± 0.98	6.87 ± 0.76	7.32 ± 0.74	7.20 ± 0.63	0.513
PLT, 10^{9}/l	227.5 ± 29.4	220.0 ± 28.8	225.8 ± 28.1	218.2 ± 24.4	0.924
N, 10^{9}/l	3.70 ± 0.58	3.73 ± 0.61	4.20 ± 0.66	3.89 ± 0.63	0.499
TBIL, μmol/l	16.4 ± 4.1	16.0 ± 2.6	15.5 ± 3.3	16.1 ± 2.9	0.972
DBIL, μmol/l	4.6 ± 1.0	4.9 ± 0.7	4.6 ± 0.9	4.7 ± 0.8	0.942
GPT, U/l	27.0 ± 12.3	26.4 ± 11.9	29.9 ± 17.1	25.6 ± 11.7	0.948
GOT, U/l	21.6 ± 11.8	21.7 ± 11.1	21.7 ± 11.9	20.2 ± 11.2	0.995
LDH, U/l	173.8 ± 19.8	169.8 ± 19.3	170.7 ± 18.3	172.5 ± 23.3	0.986
BUN, mmol/l	5.5 ± 1.2	5.5 ± 1.1	5.5 ± 0.8	5.6 ± 0.9	0.997
CR, μmol/l	89.5 ± 9.7	88.9 ± 7.2	90.5 ± 5.9	90.8 ± 7.3	0.971
GLU, mmol/l	4.7 ± 0.5	4.6 ± 0.7	4.7 ± 0.4	4.6 ± 0.5	0.962

T indicates temperature; HR, heart rate; RR, respiratory rate, SBP, systolic blood pressure; DBP, diastolic blood pressure; HB, hemoglobin; RBC, erythrocyte; PLT, platelet; N,neutrophil; TBIL, total bilirubin; DBIL, direct bilirubin; GPT, glutamate-pyruvate transaminase; GOT, glutamic-oxal(o)acetic transaminase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; CR, creatinine; GLU, glucose

therapy. The serum level of HGF before and after therapy was 22,110 ± 3247 ng/l and 22,445 ± 3730 ng/l (n = 18), respectively. No neutralizing antibody against adenovirus was detected.

General safety evaluation in follow-up

During follow-up for 12 months (from 11 to 14 months), no long-term fever or paroxysmal fever was recorded among the patients; no malignant tumour or abnormality of the biomarker for the tumour was diagnosed during the long-term follow-up, no significant abnormality of liver or renal function was found during the long-term follow-up and no significant hematocytopenia was recorded. One patient suffered from stroke 7 months after the injection of the Ad(5)-HGF, and the complication was regarded as a serious adverse event but not directly related to the treatment. Although about 5 subjects suffered from low fever on the second or third day after the injection of the Ad-HGF, they recovered to the normal body temperature on the fourth day after the injection of the Ad-HGF.

Efficacy evaluation of HGF gene therapy

To date, none of the patients had readmitted due to the angina or myocardial infarction or aggravation of the heart failure. Among the 18 patients, a significant improvement in activity tolerance was observed in the 11 patients after the about 12-month follow-up.

Discussion

The ability to biologically revascularize tissues, if proven to be safe and efficacious in large scale of controlled trials, will be an invaluable treatment for patients with diffuse disease not amenable to conventional CABG or PTCA and may be useful as an initial therapy in some individuals in place of routine therapies such as CABG and PTCA [19]. Ischemic cardiac disease that is not amenable to conventional revascularization gives a significant therapeutic challenge. Despite advances in conventional surgical and percutaneous revascularization techniques, more than 10% of patients referred to tertiary intervention centers have symptomatic coronary artery disease (CAD) that cannot be revascularized [20]. The development of strategies to deliver angiogens to ischemic myocardium for revascularization without the need for mechanical manipulation of atherosclerotic vessels is of great significance in the treatment of coronary artery disease. Preclinical studies have demonstrated that HGF can increase the number of functional arterioles and improve collateral artery growth [16, 21]. Morishita and colleagues [22] have evaluated the safety and curative effect of HGF plasmid DNA on patients

with critical limb ischemia (CLI). Their results indicate that intramuscular injection of naked HGF plasmid does not cause the severe complications and adverse effects in any patients, indicating that HGF gene transfer is safe and feasible. In this present study we have demonstrated that it is safe and feasible using an adenovirus gene-transfer vector to deliver the human hepatocyte growth factor gene to individuals with clinically significant coronary artery disease.

It has now been unequivocally demonstrated that adenovirus vector can be used to transfer and express genes to human in vivo. Local administration of low and intermediate ($<10^{11}$ PU) doses of $E1^{-}E3^{-}$ adenovirus gene transfer vectors appears to be well tolerated [23]. To achieve the objective of this study, we have chosen the parameters of blood routine, biochemical assays, cardiac and hepatonephric functions that have relevance to laboratory abnormalities observed in experimental animals receiving high dose of Ad vectors. Assessment of blood parameters suggested no systemic abnormalities related to the vector. Importantly, there was no evidence of liver function abnormalities, which was used as indications of vector dose. This is important because the liver is a major site of Ad vector-induced inflammation at high doses in some studies in experimental animals [24]. One explanation for lack of systemic toxicity in the human studies is that the vector preparations used in clinical trails are highly purified. In addition, there was no evidence of systemic immunity-related toxicity in any patient, including no immediate anaphylactic responses, vasculitis, or renal damage.

There was no evidence of increases in arrhythmias or ST/T wave changes assessed by Holter and ECG monitoring, and also no evidences of excess deranged angiogenesis, myocardial edema or pericardial effusions.

To the best of our knowledge, this is the first study about the phase I assessment of direct intracoronary administration of Ad(5)-HGF to individuals with clinically significant severe coronary artery disease. These observations are consistent with the assessment of the safety of human VEGF121 cDNA plasmids applied to the human myocardium by intracoronary administration [19].

One of the major limitations of application of adenoviruses as gene therapy vectors is the presence of neutralizing anti-Ad antibody titers in various populations. However, the presence of neutralizing anti-Ad antibody titers in the serum of patients is not necessarily correlated with transgene expression in heart by intracoronary adenovirus administration. We analyzed the neutralizing anti-Ad antibody before and after single administration of Ad-HGF, and no specific neutralizing anti-Ad antibodies were observed.

However, the main limitation of this present study was that we did not report the effects of the Ad-HGF on left ventricular function/ejection fraction. Another limitation is the fact that there appears to be an extremely large degree of variability in our data, and that, as a result, that our sample size might be too small to capture significant differences in the measured variables. Therefore, the results of the present study need to be confirmed in a study with a substantially larger sample size, and a great deal of additional work is necessary before administration of Ad-HGF can be recommended for clinical practice.

In conclusion, we have demonstrated that it is safe and feasible using an adenovirus gene-transfer vector to deliver the human hepatocyte growth factor gene to the individuals with clinically significant coronary artery disease.

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