Cell Biology

Circulating Immunoreactive Endothelin-1 in Patients Undergoing Percutaneous Transluminal Coronary Angioplasty: Effect of Intracoronary Beta-Irradiation and Prolonged Perfusion Balloon Inflation

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Purpose: This study assessed whether intracoronary brachytherapy after percutaneous transluminal coronary angioplasty (PTCA) affects the peripheral venous plasma endothelin-1 (ET-1) levels and whether changes in ET-1 levels are associated with 6-month restenosis.

Methods: Thirteen and 12 study patients who received post-PTCA 20-and 14-Gray (Gy) intracoronary β-irradiation, respectively, were enrolled. Six control patients received 5 minutes of post-PTCA inflation with a perfusion balloon catheter. Blood samples for plasma ET-1 levels measurements were collected immediately before, at 1 hour and 24 hours after PTCA procedures from the antecubital vein.

Results: Compared to pre-PTCA, ET-1 levels of the 14-Gy and 20-Gy irradiation groups were significantly higher at 1-hour post-PTCA and returned to baseline after 24 hours (8.23 ± 3.09 vs. 10.21 ± 4.84 vs. 7.91 ± 3.97 pg/ml; p = 0.04 and 8.53 ± 3.25 vs. 16.08 ± 10.45 vs. 9.75 ± 3.51 pg/ml; p = 0.004, respectively). There were no significant changes in ET-1 levels measured in the control group (9.92 ± 4.42 vs. 7.51 ± 2.08 vs. 7.79 ± 4.60 pg/ml; p = 0.144). The transient increase of ET-1 levels was strongly associated with higher inflation time (multiple linear regression analysis; p = 0.002). The 6-month angiographic restenosis rates of the control, 14-Gy irradiation and 20-Gy irradiation groups, respectively, were 50%, 33% and 23% (p = 0.50) and were not associated with transient increases of ET-1 levels.

Conclusion: A transient increase in the peripheral plasma ET-1 levels was caused by lengthy perfusion balloon inflation after PTCA during intracoronary irradiation with Re-188, and this increase was not associated with an increased 6-month restenosis rate.

Key Words: β-irradiation • Endothelin-1 • Percutaneous transluminal coronary angioplasty • Restenosis

INTRODUCTION

Increased plasma endothelin levels have been reported during and after percutaneous transluminal coronary angioplasty (PTCA).[1-9] This has been attributed to mechanical trauma of the local endothelial cells.[1-5] Previous studies have demonstrated that endothelin is a comitogen for vascular smooth muscle cell proliferation and a factor stimulating extracellular matrix production at the site of vascular injury.[10-16] Hence, a causal re-

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lationship between endothelin levels and restenosis after PTCA may exist.

The success of PTCA with baremetal stents is hindered by development of restenosis in up to 41% of cases within the first 6 months of the initial procedure.[17,18] Intracoronary radiation using either γ- or β-rays has been proven to reduce the 6-month restenosis rate in many studies.[19-24] The mechanism of intracoronary brachytherapy remains controversial. An in-vitro study showed a decrease of endothelin-1 (ET-1) secretions in the γ-irradiated cultured rat vascular smooth muscle cells.[25] Thus, this study investigated whether intracoronary brachytherapy with liquid-form Rhenium-188 (Re-188) delivered by a perfusion balloon catheter after PTCA affects peripheral venous plasma ET-1 levels: that is, this study attempted to define whether the level of peripheral plasma ET-1 is a restenosis marker. To easily obtain samples for analysis, antecubital venous blood rather than coronary sinus or arterial blood was taken.

MATERIALS AND METHODS

Procedure

Twenty-five patients were enrolled in this study. Each received intracoronary β-irradiation with liquid-form Re-188 delivered by a Lifestream perfusion balloon catheter (Advanced Cardiovascular Systems, Santa Clara, California). Twelve of the 25 patients received intracoronary β-irradiation 14-Gy and 13 received 20-Gy intracoronary β-irradiation after PTCA. Six control patients received 5-minute post-PTCA inflation with a perfusion balloon catheter. No patient received stent implantation or a procedure with a device other than balloon. Blood samples for plasma ET-1 levels measurements were collected immediately before, at 1 hour and 24 hours after PTCA procedures from the antecubital vein. This study was approved by the Ethics Committee of our institution.

The enrollment criteria were as follows: aged 50 years or older; clinically required balloon angioplasty of a native coronary artery (either de novo or post-PTCA restenotic lesion); and a target lesion with a reference vessel between 2.5 and 3.5 mm in diameter and lesion length ≤ 25 mm. Patients were excluded if they met the following criteria: final angiographic residual stenosis by on-line quantitative coronary analysis (QCA) was greater than 30%; a stent had been implanted; angiographic evidence of thrombus in the target lesion; pre-menopausal; previous thoracic therapeutic radiation; advanced renal failure (creatinine greater than 3.0); left ventricular ejection fraction < 25%; evolving myocardial infarction or had suffered from myocardial infarction within 72 hours; and had received thrombolytic or glycoprotein IIb/IIIa inhibitors within the previous 48 hours. At 1 month and 6 months after the PTCA procedure, data for recurrent ischemic symptoms, death, target vessel myocardial infarction, or revascularization of the treated vessel was obtained from charts, outpatient visits or telephone interviews. All patients were admitted to Chang Gung Memorial hospital at roughly 6 months after the procedure for repeat coronary angiography.

Patients were pretreated with aspirin (100 mg/day). An intravenous or intracoronary bolus of 10,000 I.U. heparin was administered prior to insertion of a 0.014 inch guidewire into the target coronary artery. Visual estimation or quantitative coronary angiography of the reference vessel diameters determined PTCA balloon size. Gradual, incremental increases of the PTCA balloon pressure or size were applied to achieve less than 30% residual stenosis by on-line QCA. Involved lesions were treated successfully where residual stenosis was less than 30% by on-line QCA. After successful PTCA, media-to-media measurements at the index study were obtained by intravascular ultrasonography (IVUS) for dosing the radiation. Irradiation and control procedures were conducted after successful PTCA without stenting or use of other devices. A perfusion balloon dilatation catheter was utilized for both the Re-188 and control groups to deliver, respectively, the irradiation and the diluted contrast (placebo). Study patients received either 14-Gy or 20-Gy intracoronary β-irradiation at 0.5 mm tissue depth after PTCA. The perfusion balloon sizes for delivery of Re-188 isotope were within ± 0.5 mm of the reference vessel diameter by IVUS. The balloon was manually inflated with the Re-188 solution at roughly 3 atmospheres.

Measurements of plasma ET-1 levels

Plasma ET-1 levels were measured by collecting blood samples in a test tube containing anticoagulant ethylene-diamine-tetraacetic acid. Samples were immediately centrifuged at 2,000 g for 15 min, and the plasma

was stored at -70 °C until further analysis. The ET-1 concentrations of human plasma samples were quantified with a commercially available sandwich-enzyme immunoassay kit (R&D Systems: Minneapolis, MN). Extraction procedures were performed as follows. First, 500 μl of plasma was added to 750 μl of extraction solvent (acetone: 1N HCl: water = 40:1:5) and mixed by inversion. The mixture was centrifuged for 20 minutes at 2000 g. The supernatant was decanted and then dried in a centrifugal evaporator (minimum drying time 4 hours at 37 °C). The reconstituted pellet was vertex for 30 seconds in 250 μl sample diluent and stored overnight at -4 °C. All samples and standards were run in duplicate. The sensitivity of the assay was determined at 0.6 pg/mL.

Statistical analysis

All data were expressed as means value ± SD. Changes in variables within groups were evaluated by an analysis of variance for repeated measures and Tukey's post hoc multiple comparisons where appropriate. Analysis of frequency counts were performed with a Chi-square test or Fisher's exact test for small samples. Comparison of change in parameter concentrations within each group and among 3 groups were examined by independent *t* test and repeated measures of analysis of variance (ANOVA), respectively. Linear regression analyses were used to determine the relationship between variables (age, sex, clinical presentations, groups and inflation time) and the ET-1 level at 1 hour after PTCA procedure. Comparisons of increases in ET-1 levels at 1 hour after PTCA procedure between patients with restenosis and those without restenosis were measured by Wilcoxon rank sum test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

There were no significant differences in the clinical and angiographic characteristics between the control and study groups (Tables 1-2). In the Re-188 irradiation group, the prescribed dose of 14-Gy or 20-Gy at 0.5 mm tissue depth was delivered with liquid Re-188 via a perfusion dilatation balloon catheter. Mean dwell time (perfusion balloon inflation time) for the14-Gy irradiation group (n = 12) was 346 ± 122 seconds (range, 175-565 seconds) (Table 3). The mean dwell time for the 20-Gy irradiation group (n = 13) was 897 ± 219 seconds (range, 620-1396 seconds) (Table 3). Dwell time (perfusion balloon inflation time) for the control group was 300 seconds (Table 3).

The 1-hour post-PTCA ET-1 levels in the 14-Gy irradiation group were significantly higher than pre-PTCA levels and returned to baseline after 24 hours (8.23 ±

Table 1. Baseline characteristics of patients*

	14 Gy	20 Gy	Control
Number (no.) of patients	12	13	6
Male, n (%)	8 (67)	10 (77)	5 (80)
Age in years	62.6 ± 6.5	65.1 ± 4.4	65.7 ± 9.3
Diabetes mellitus, n (%)	2 (17)	5 (38)	3 (50)
Hypertension, n (%)	7 (58)	6 (46)	4 (67)
Smoker, n (%)	1 (9)	3 (29)	3 (50)
Serum cholesterol > 200 mg%, n (%)	6 (50)	6 (46)	5 (80)
Left ventricular ejection fraction (%)	70.6 ± 14.5	67.7 ± 15.4	71.3 ± 12.5
Previous myocardial infarction, n (%)	4 (33)	7 (54)	2 (33)
Stable angina, n (%)	7 (58)	6 (46)	1 (20)
Unstable angina, n (%)	5 (42)	7 (54)	5 (80)
3-vessel disease, n (%)	6 (50)	4 (30)	4 (67)
2-vessel disease, n (%)	5 (42)	8 (61)	1 (17)
Left anterior descending artery, n (%)	4 (34)	3 (23)	1 (17)
Left circumflex artery, n (%)	7 (58)	6 (46)	2 (33)
Right coronary artery, n (%)	1 (8)	4 (31)	3 (50)

*Plus-minus values are means + SD; p was not significant for any factor.

Table 2. Baseline and post-procedure quantitative angiographic results*

	Re-188 14 Gy	Re-188 20 Gy	Control	p value
No. of lesions	12	13	6	
Pre procedure				
Reference diameter, mm	2.85 ± 0.36	2.59 ± 0.51	2.81 ± 0.33	0.31
Average MLD, mm	0.73 ± 0.29	0.69 ± 0.28	0.59 ± 0.23	0.60
% Diameter stenosis	74.0 ± 8.7	74.2 ± 8.6	78.5 ± 6.6	0.51
Post-procedure				
Reference diameter, mm	2.90 ± 0.28	2.88 ± 0.34	3.02 ± 0.34	0.66
Average MLD, mm	2.39 ± 0.29	2.36 ± 0.37	2.62 ± 0.52	0.37
% Diameter stenosis	17.4 ± 8.6	18.8 ± 9.4	12.0 ± 9.1	0.31

MLD, minimal luminal diameter; *plus-minus values are means ± SD; p was not significant for any factor.

Table 3. Plasma ET-I levels in antecubital venous blood and perfusion balloon inflation times*

	Re-188 14 Gy	Re-188 20 Gy	Control	p-value
Perfusion balloon inflation time	389 ± 186 sec	848 ± 284 sec	300 ± 0 sec	< 0.0001
Range	175-697 sec	342-1396 sec	300-300 sec	
ET-1 levels				
Before PTCA (pg/ml)	8.23 ± 3.09	8.53 ± 3.25	9.92 ± 4.42	0.609
1-hour after PTCA/irradiation (pg/ml)	10.21 ± 4.84	16.08 ± 10.45	7.51 ± 2.08	0.051
24-hour after PTCA/irradiation (pg/ml)	7.91 ± 3.97	9.75 ± 3.51	7.79 ± 4.60	0.428
p-value	0.04	0.004	0.144	

ET-1, endothelin-1; PTCA, percutaneous transluminal coronary angioplasty; *plus-minus values are means ± SD; p-values were evaluated using independent *t* test or repeated measures of ANOVA.

3.09 vs. 10.21 ± 4.84 vs. 7.91 ± 3.97 pg/ml; p = 0.04) (Table 3). In the 20-Gy irradiation group, the 1-hour post-PTCA ET-1 levels were substantially higher than pre-PTCA levels and returned to the baseline after 24 hours (8.53 ± 3.25 vs. 16.08 ± 10.45 vs. 9.75 ± 3.51 pg/ml; p = 0.004) (Table 3). The changes in ET-1 levels of the control patients measured before, at 1 hour and 24 hours were not significant (9.92 ± 4.42 vs. 7.51 ± 2.08 vs. 7.79 ± 4.60 pg/ml; p = 0.144) (Table 3). The transient increase in ET-1 levels was strongly associated with lengthy inflation time (multiple regression coefficient, r = 0.019; p = 0.002) (Table 4).

Reference vessel diameters of the control group, 14-Gy irradiation group and 20-Gy irradiation group at 6 months follow-up were, respectively, 2.96 ± 0.37 mm, 3.08 ± 0.65 mm and 3.04 ± 0.47 mm (p = 0.56) (Table 5). The minimal luminal diameter (MLD) of the control group, 14-Gy irradiation group and 20-Gy irradiation group were, respectively, 1.67 ± 0.83 mm, 1.90 ± 1.19 mm and 1.41 ± 0.94 mm (p = 0.25) at 6-month follow-up (Table 5). The percent diameter stenosis of the control group, 14-Gy irradiation group and 20-Gy irradiation groups were, respectively, 54.8 ± 29.5%, 42.7 ± 28.9% and 40.6 ± 28.6% (p = 0.19) (Table 5). The 6-month angiographic restenosis rates of the control group, 14-Gy irradiation group and 20-Gy irradiation group, respectively, were 50% (3 of 6), 33% (4 of 12) and 23% (3 of 13) (p = 0.50) (Table 5). Six-month angiographic restenosis rates for the control, 14- and 20-Gy irradiation groups were not associated with transient increases of ET-1 levels (Table 6).

DISCUSSION

Endothelin is a 21-amino-acid peptide with vaso-

Table 4. Linear regression analyses of ET-1 levels 1-hour after PTCA/irradiation with other variables

	Univariate regression coefficient	p-value	Multiple regression coffecient	p-value
Age	0.053	0.827	-0.016	0.933
Sex	3.872	0.250	1.361	0.641
Clinical presentation (stable and unstable angina)	-1.791	0.552	-2.907	0.264
Inflation time	0.016	< 0.0001	0.019	0.002
3 groups	4.534	0.017	-1.163	0.606

Table 5. Six-month quantitative angiographic results*

	Re-188 14 Gy	Re-188 20 Gy	Control	p value
No. of lesions at six months	12	13	6	
Reference diameter, mm	2.96 ± 0.37	3.08 ± 0.65	3.04 ± 0.47	0.56
Average MLD, mm	1.67 ± 0.83	1.90 ± 1.19	1.41 ± 0.94	0.25
% Diameter stenosis	42.7 ± 28.9	40.6 ± 28.6	54.8 ± 29.5	0.19
Restenosis, n (%)	4 (33)	3 (23)	3 (50)	0.50

*Plus-minus values are means ± SD; p was not significant for any factor.

constricting properties 10 times those of angiotensin II.[26,27] Three structurally and pharmacologically distinct isopeptides have been identified, however, only ET-1 is synthesized by vascular endothelial cells.[26] Although most vasoactive substances are released in surges following local mechanical or chemical stimulation, ET-1 is released slowly via ET_A or ET_B receptor-mediated mechanisms in membranes and activates intracellular protein C that produces smooth muscle contraction.[26-30] Upregulation of ET_B receptors has been demonstrated in the vascular smooth muscle cells from the subcutaneous tissue of the abdomen of the patients with ischemic heart disease.[31] Elevated ET-1 levels are associated with coronary artery disease, pulmonary hypertension and congestive heart failure.[32-34] After acute myocardial infarction, high plasma ET-1 levels are predictive of 1-year mortality.[35] Previous studies showed that ET-1 levels were elevated immediately after PTCA at the coronary sinus and even more so in the dilated coronary artery distal to the site of the PTCA.[3,4,7] The ET-1 mediates (or co-mediates) mitogenesis and thus, contributes to the proliferation of vascular smooth muscle cells.[11,12] Chronically elevated plasma and/or local endothelin levels, can lead to enhanced proliferation of the blood vessel wall.[16,19] A considerable effort has recently been made to better define the role of endothelin and its two-receptor subtypes in restenosis.[13-16,37-39] Several different endothelin receptor antagonists have been tested in different restenosis models in rats and pigs.[40-43] These studies have shown that both in rat and in pig models, blockage of the ET_A receptor causes reduction of restenosis.[40-43]

Table 6. Comparison of the increase of ET-1 after 1 hour between patients with restenosis and those without restenosis*

	Restenosis	n	ET-1 increase	p value
Re-188 14 Gy	Yes	6	3.52 ± 4.71	0.30
	No	6	0.45 ± 0.87	
Re-188 20 Gy	Yes	2	3.09 ± 3.55	0.37
	No	11	8.37 ± 9.32	
Re-188 14 Gy+20 Gy	Yes	8	3.41 ± 4.21	0.83
	No	17	5.57 ± 8.35	
Control	Yes	3	-1.15 ± 1.26	0.66
	No	3	-3.67 ± 3.37	

*Plus-minus values are means ± SD; p was not significant for any factor by Wilcoxon rank sum test.

All the 25 patients in our study were non-systemically taken from the "In Taiwan Radiation in Prevention of Post-Pure Balloon Angioplasty Restenosis" (TRIPPER) study.[24] In the TRIPPER study, 40 patients underwent 14 Gy irradiation and 15 patients underwent 20 Gy irradiation at 0.5 mm tissue depth after pure balloon

angioplasty (POBA). Thirty control patients received a 5-minute inflation with a perfusion balloon catheter after POBA. Six-month angiographic restenosis rates were 49% in the 14 Gy group, 20% in the 20 Gy group, and 57% in the control group (20 Gy vs. control; $p = 0.05$). Six-month angiographic restenosis rates were 49% in the 14 Gy group, 20% in the 20 Gy group, and 57% in the control group (20 Gy vs. control; $p = 0.05$). In our study, the 6-month angiographic restenosis rates of the 14-Gy irradiation group, 20-Gy irradiation group and control group (33%, 23% and 50%, respectively) were not significantly different due to smaller number of patients enrolled.

Clinical studies have demonstrated that a substantial reduction in the rate of post-PTCA restenosis was achieved in patients receiving catheter-based γ- and β-radiation.[22,23] However, the complex mechanisms and pathophysiology of restenosis are not fully understood. In the cultured rat vascular smooth muscle cells study conducted by Zhong et al., cobalt-60 γ-radiation at doses of 14 and 25 Gy decreased the secretions of ET by 27.3% ($p < 0.01$) and 58% ($p < 0.01$), respectively.[25] Our study did not show a decrease in peripheral plasma ET-1 levels by intracoronary β-radiation therapy with 14 and 20 Gy as compared to the control. The Lifestream perfusion balloon catheter can, according to its manufacturer, delivers a distal coronary flow rate of 37-40 ml/min. Although clinically significant ischemia can be suppressed in the majority of patients with a coronary flow rate of 20-40 ml/min, changes to myocardial lactate extraction and free radical production indicated myocardial ischemia during perfusion balloon angioplasty.[44] Hence, the increase of the peripheral venous plasma ET-1 levels likely resulted from transient prolonged partial occlusion of the coronary artery and prolonged complete occlusion of its side-branches. Moreover, elevation of peripheral plasma post-PTCA ET-1 level was transient and strongly associated with the inflation time. The suppression effect of the irradiation on the secretion of ET-1 might be masked by the more pronounced effect of the prolonged perfusion balloon inflation. The transient high peripheral ET-1 levels were not associated with an increased restenosis rate. However, local ET-1 levels at the target vessel might be persistently elevated but not high enough to affect peripheral ET-1 levels. Local ET-1 levels can likely yield a better definition of the role of ET-1 in post-PTCA restenosis.

CONCLUSION

A transient increase in the peripheral plasma ET-1 levels was caused by lengthy perfusion balloon inflation after PTCA during intracoronary irradiation with Re-188, and this increase was not associated with an increased 6-month restenosis rate.

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