Influence of Postangioplasty β-Irradiation on Endothelial Function in Porcine Coronary Arteries

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Background—Postangioplasty (PTCA) intracoronary radiation therapy (ICRT) has been demonstrated to limit restenosis. The consequences of these procedures on coronary reactivity are unknown.

Methods and Results—Porcine coronary arteries were studied after PTCA immediately (n=5) and 6 weeks (n=5) after ICRT (n=5 and 5, respectively), after combined PTCA+ICRT (n=5 and 7, respectively), and after no intervention (n=11). A 3-cm-long source train of Sr/Y^{90} was used in vivo to deliver 16 Gy at a depth of 2 mm from the source center, as used in clinical trials. Arterial rings were mounted on myographs to record isometric tension. After achieving steady-state contraction to depolarizing physiological solution containing 40 mmol/L KCl, measured baseline tension was significantly elevated immediately after all interventions. It returned to normal levels 6 weeks after PTCA and ICRT alone but was significantly reduced if combined. Active contractions induced by 40 mmol/L KCl were maintained after combined therapy both immediately after and at 6 weeks. In these depolarizing conditions, nitric oxide–dependent relaxation to substance P was trivial after PTCA+ICRT and reduced after ICRT, whereas in the presence of physiological solution and N^{ω}-nitro-L-arginine, substance P–induced relaxation was reduced after PTCA and abolished after PTCA+ICRT 6 weeks after intervention. In rings without endothelium, the relaxation mediated by sodium nitroprusside (0.1 μmol/L) was reduced immediately after PTCA and at 6 weeks.

Conclusions—PTCA+ICRT altered the passive mechanical properties of porcine coronary arterial wall. Furthermore, at 6 weeks, receptor-operated release of endothelium-derived nitric oxide and endothelium-derived hyperpolarizing factor was reduced by ICRT and PTCA alone, respectively, and was prevented by their combination. **(*Circulation*. 2000;101:1430-1435.)**

Key Words: angioplasty ■ radioisotopes ■ endothelium

Intracoronary radiation therapy (ICRT) after angioplasty has recently been implemented to prevent and treat restenosis. The anatomic antirestenotic effects of these interventions have been reported for the short and medium term.[1–4] Longer-term consequences of these interventions on the patency of the coronary vessels are currently under investigation. Animal studies have shown that irradiation produces immediate endothelial damage, with uniform impairment of response to acetylcholine, serotonin, and nitroglycerin demonstrated immediately after radiation damage.[5–11] In vitro follow-up at 6 months of irradiated rat aorta revealed impairment of the response to nitroglycerin and acetylcholine,[5] whereas in vivo follow-up of irradiated porcine coronary arteries demonstrated an impaired response to nitroglycerin with a preserved response to acetylcholine at 4 weeks.[6]

Prolonged impairment of endothelial function may reflect an increased risk of delayed thrombosis and accelerated atherosclerosis beyond that expected after standard percutaneous transluminal coronary angioplasty (PTCA). Conversely, the rapid return of normal endothelial function would be supportive of the doses used in clinical trials with an associated low restenosis rate. Furthermore, reports of aneurysm and pseudoaneurysm formation in the first postangioplasty radiation study,[12] in which higher-than-expected doses were delivered to certain aspects of the arterial wall, raises the possibility that significant vessel wall death took place at those sites. No studies thus far have reported the physiological response of vessels after the combined injury of angioplasty and irradiation.

The aim of this study was to investigate the consequences of ICRT after PTCA on endothelial function and the reactivity of normal pig coronary arteries isolated immediately and 6 weeks after the intervention. This study was performed on isolated coronary vessels to allow for a precise pharmacological characterization of the endothelium-derived factors in-

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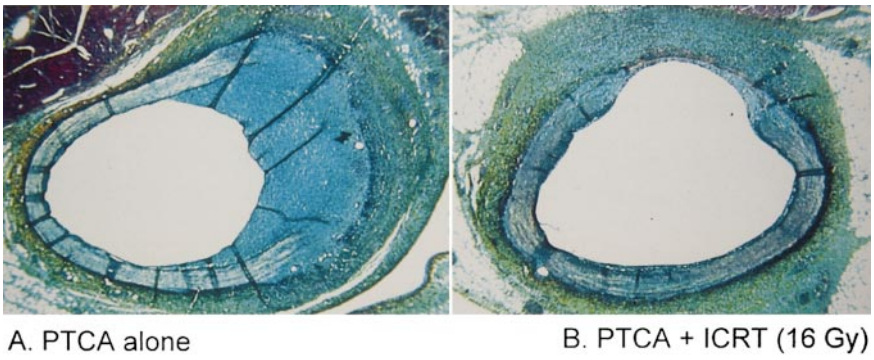

Figure 1. Porcine coronary arteries 6 weeks after angioplasty with significant neointimal formation at site of internal elastic lamina fracture (A) and 6 weeks after angioplasty+radiation therapy, demonstrating no neointimal formation but evidence of vessel wall thinning at site of internal elastic lamina fracture (B) (Movat pentachrome stain).

volved in the regulation of vascular reactivity post PTCA with or without ICRT.

Results

Twenty-two left anterior descending (LAD) and 21 left circumflex (Cx) arteries were used in this study, for a total of 43 separate porcine coronary arteries. Six arteries were studied as controls from animals that had undergone no irradiation, and another 5 vessels were from animals having undergone treatment in another vessel. There was no difference between these 2 groups, and they are subsequently considered as 1 control group (n=11). The remaining arms were the immediate and 6-week post-PTCA, 5 vessels each; immediate and 6-week post-ICRT, 5 vessels each; and immediate and 6-week post–combined PTCA+ICRT, 5 and 7 vessels, respectively. No difference between the response of the LAD and Cx arteries was noted.

Histology

In the balloon-injured arteries, at the site of internal elastic membrane (IEM) rupture, there was maximal intimal proliferation, as shown in Figure 1. This was composed of predominantly smooth muscle cells. Twenty percent to 50% of the IEM circumference was ruptured. Conversely, in the vessels that underwent angioplasty followed by irradiation, there was limited neointima and evidence of vessel wall thinning despite equivalent IEM rupture. These results are consistent with those previously reported describing and quantifying the effects of both β- and γ-irradiation after balloon injury in a porcine coronary model.[13–15]

Reactivity Study

Baseline Tension

Figure 2 represents the resting baseline tension of isolated coronary arterial rings. This is defined as the passive tension that promotes a maximal contractile response to a depolarizing solution containing 40 mmol/L KCl.

After all immediate interventions, baseline tension was significantly increased ($P<0.05$). Resting baseline tension of isolated rings 6 weeks after PTCA or ICRT alone had returned to normal levels; however, it was significantly reduced ($P<0.05$) at 6 weeks after their combination.

Contractile Responses

Immediate PTCA alone decreased the amplitude of the contraction induced by 40 mmol/L KCl, with no improvement at 6 weeks (Figure 3). ICRT alone potentiated this response in rings with an intact endothelium immediately ($P<0.05$) but not after 6 weeks. PTCA+ICRT had no effect on this response.

Contraction induced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (40 μmol/L) was potentiated by endothelial denudation (Figure 4). This response was maintained after immediate ICRT

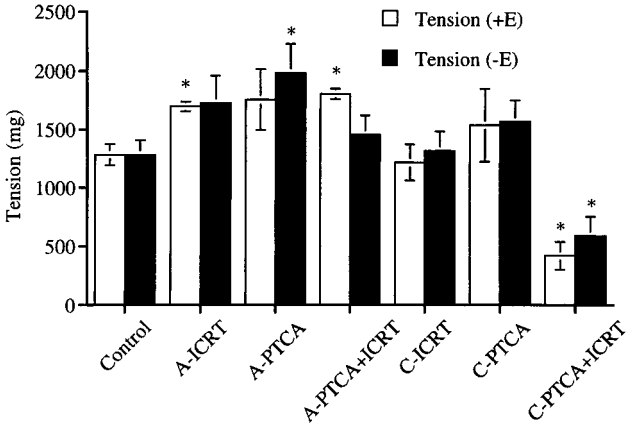

Figure 2. Effects of PTCA and ICRT alone or combined on resting tension of isolated rings of coronary arteries with (+E) or without (−E) endothelium. Data are expressed as mean±SEM. *$P<0.05$ compared with control rings. A, Acute (immediate); C, chronic (long-term).

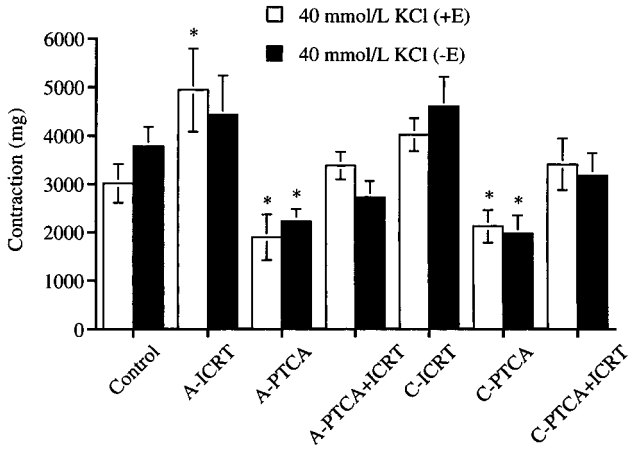

Figure 3. Effects of PTCA and ICRT alone or combined on contraction induced by 40 mmol/L KCl-physiological solution of isolated rings of coronary arteries with (+E) or without (−E) endothelium. Data are expressed as mean±SEM. *$P<0.05$ compared with control rings. A, Acute (immediate); C, chronic (long-term).

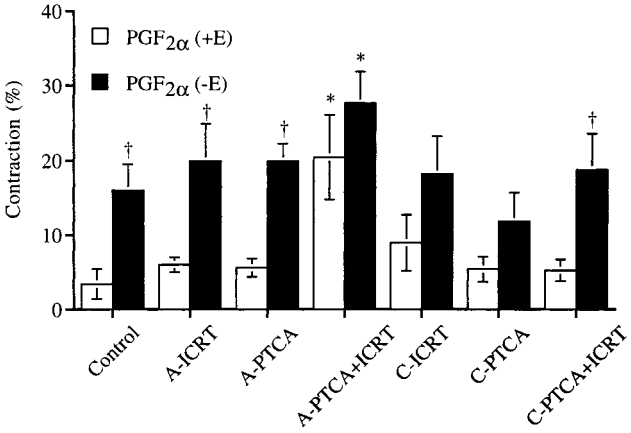

Figure 4. Effects of PTCA and ICRT alone or combined on the contraction induced by $PGF_{2\alpha}$ (40 μmol/L) of isolated rings of coronary arteries with (+E) or without (−E) endothelium. Data are expressed as mean±SEM. *$P<0.05$ compared with control rings (n=11); †$P<0.05$ compared with +E. A, Acute (immediate); C, chronic (long-term).

or PTCA alone. Immediately after combination of the 2 therapies, however, potentiation of $PGF_{2\alpha}$-induced contraction occurred irrespective of the presence of the endothelium. After 6 weeks, $PGF_{2\alpha}$ contraction potentiation was limited to the endothelium-denuded rings only and thus was similar to the control response.

Endothelium-Dependent Relaxation

In rings preconstricted with $PGF_{2\alpha}$ (40 μmol/L), substance P (0.1 μmol/L) induced a complete endothelium-dependent relaxation (Figure 5). This relaxation was decreased after all interventions immediately and did not recover after 6 weeks.

To identify the respective contributions of release of an endothelium-derived hyperpolarizing factor (EDHF) and nitric oxide (NO) in the alteration of this endothelium-derived relaxing factors (EDRF)-dependent effect, we investigated the relaxant effects of substance P in 2 different experimental

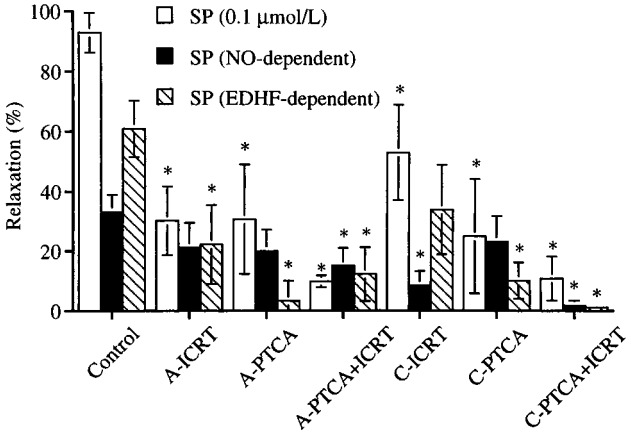

Figure 5. Effects of PTCA and ICRT alone or combined on the endothelium-dependent relaxation mediated by substance P (SP, 0.1 μmol/L) of isolated rings of coronary arteries either preconstricted by $PGF_{2\alpha}$ (40 μmol/L), 40 mmol/L KCl-physiological solution (NO-dependent), or $PGF_{2\alpha}$ (40 μmol/L) after NO synthase inhibition with N^{ω}-nitro-L-arginine (100 μmol/L) (EDHF-dependent). *$P<0.05$ compared with control rings. A, Acute (immediate); C, chronic (long-term).

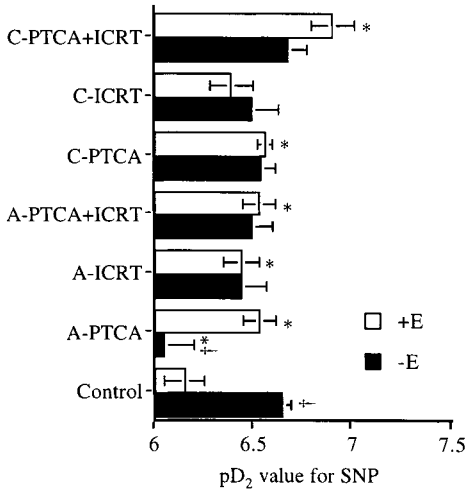

Figure 6. Sensitivity (pD_2) to SNP of isolated rings of coronary arteries with (+E) or without (−E) endothelium preconstricted with 40 mmol/L KCl-physiological solution. Experiments were performed in control rings, after PTCA, after ICRT, and after PTCA+ICRT. Data are expressed as mean±SEM. *$P<0.05$ compared with control rings; †$P<0.05$ compared with +E. A, Acute (immediate); C, chronic (long-term).

conditions. In depolarized conditions (tone induced by 40 mmol/L KCl and in the presence of indomethacin), substance P induced an NO-dependent relaxation that was significantly decreased only after immediate PTCA+ICRT compared with control. After 6 weeks, this NO-dependent relaxation was only trivial.

Although unchanged immediately, 6 weeks after ICRT, substance P–induced NO-dependent relaxation was reduced. PTCA, both immediate and long term, did not significantly modify the NO-dependent relaxation to substance P.

A potent endothelium-dependent, EDHF-mediated relaxation was induced by substance P (Figure 5). This relaxation was observed in control rings preconstricted with $PGF_{2\alpha}$ (40 μmol/L) combined with NO synthase inhibition by N^{ω}-nitro-L-arginine (100 μmol/L) and in the presence of indomethacin. This relaxation was reduced immediately after all interventions. After 6 weeks, the EDHF-dependent relaxation mediated by substance P was not significantly reduced by ICRT alone. It remained lower after PTCA alone and was eliminated after the combination of PTCA+ICRT.

Endothelium-Independent Relaxation

The sensitivity (pD_2) to sodium nitroprusside (SNP) (1 nmol/L to 10 μmol/L) of isolated rings preconstricted with a depolarizing solution (40 mmol/L KCl) was measured (Figure 6). Contrary to control, the sensitivity to SNP of treated rings was unaffected by endothelial denudation except immediately after PTCA alone, in which the pD_2 value to SNP decreased. This opposite effect was absent 6 weeks after PTCA. Finally, the sensitivity to SNP was higher after long-term PTCA+ICRT.

Discussion

The objectives of this study were to investigate in vitro the consequences of ICRT after PTCA on coronary reactivity and its endothelium-dependent regulation. The results, summa-

Summary of Results 6 Weeks After PTCA and ICRT Alone or Combined

	ICRT	PTCA	PTCA+ICRT
Neointima formation	N/A	+++	−−−
Passive mechanical properties	=	=	−−−
Contraction to K^+	=	−	=
Contraction to $PGF_{2\alpha}$	=	=	=
Endothelium-dependent relaxation	−	−−	−−−
Endothelium-independent relaxation	=	=/−	+

+ indicates Increase; −, decrease; =, similar to the control response; N/A, not applicable.

rized in the Table, reveal that PTCA+ICRT has 2 important consequences: (1) it alters the passive mechanical properties of normal porcine coronary arterial wall, and (2) the relaxation mediated by endothelium-derived NO and EDHF are prevented without affecting smooth muscle contractility.

Intracoronary radiation therapy has been used in patients in an attempt to prevent restenosis. At doses similar to that used in this study, significant reduction of neointimal formation was attained.[4] However, reports of aneurysm and pseudoaneurysm formation in the first postangioplasty radiation study,[12] in which higher-than-expected doses were used (up to a theoretical maximum of 92.5 Gy), raise the possibility that significant vessel wall death occurred when the combination of PTCA and ICRT was used. Despite the use of significantly lower doses as in this study, previously reported quantitative histomorphometric analyses showed a thinning of the arterial wall at the site of IEM rupture.[13–15] In distinction to PTCA alone, its combination with ICRT reduced the width of the media at the IEM rupture site. These morphological changes were associated with a decrease in the passive mechanical properties of the coronary arterial wall (Figure 2). Arterial rings were stretched sequentially to a level that sustained a maximal contractile response induced by high K^+ solution. As shown in Figure 3, PTCA+ICRT did not alter the amplitude of the contractile response. However, resting basal tension was severely reduced in this group (Figure 2). The baseline tension is normally independent of smooth muscle cell contraction in isometric myographs. Indeed, addition of a supramaximal concentration of SNP in Ca^{2+}-free physiological solution does not decrease the tension of arterial rings below the level of the resting basal tension. Rather, it is the elastic component of the arterial wall that is responsible for this tension.[16] Dissection after PTCA alone, either immediately or long term, had no effect on the resting basal tension, suggesting that the dissection of the intimal layer does not weaken the arterial wall. Thus, after PTCA, ICRT has multiple effects on the arterial wall structure, including a reduction in smooth muscle mass and a loss of elasticity not evidenced when these treatments are delivered in isolation or immediately.

Contractile responses to high K^+ and $PGF_{2\alpha}$ were maintained 6 weeks after PTCA+ICRT. Only PTCA alone significantly reduced the contraction induced by high external K^+. There is therefore a discrepancy between the results obtained with 2 different stimuli after PTCA that was not modified after 6 weeks of recovery. This discrepancy may be explained by the difference in amplitude of the contraction induced by the 2 stimuli: PTCA alone reduced the ability of the isolated vessels to fully constrict (high K^+), whereas a lower contraction induced by $PGF_{2\alpha}$ was maintained. This suggests that PTCA alone reduces the maximal contractile response. However, acute ICRT prevented the decreased response to high K^+ after PTCA. This is most likely due to the direct effect of ICRT on the endothelium. Indeed, as seen in Figure 3, there is a strong potentiation of the contractile response to high K^+, suggesting that ICRT immediately reduces the endothelium-dependent inhibitory effect on coronary contractility.

Interestingly, $PGF_{2\alpha}$-induced contractions of denuded arterial rings were not affected by the different procedures (Figure 4). Removal of the endothelium potentiated the contraction mediated by $PGF_{2\alpha}$, although this potentiation was not significant 6 weeks after ICRT or PTCA alone. This suggests that the basal release of EDRFs significantly prevented $PGF_{2\alpha}$-induced contractions.

The contractile response induced by $PGF_{2\alpha}$ was increased after acute PTCA+ICRT irrespective of the presence or absence of endothelium. Thus the endothelium-dependent regulation of this contractile response was obliterated. This may be due either to the release of vasoconstricting factors counterbalancing the relaxant effects of EDRF or by a lack of basal EDRF production. This effect was transitory, suggesting recovery of some endothelium-dependent regulation after 6 weeks. The endothelium had not been entirely removed by the PTCA+ICRT because substance P still induced a relaxation immediately, albeit significantly reduced (Figure 5).

Substance P stimulated the release of EDRF that produced a maximal relaxation of control coronary arterial rings precontracted by $PGF_{2\alpha}$ in the presence of indomethacin (Figure 5). The contribution of NO appears to be less than that of EDHF. A similar observation has been recently reported for acetylcholine-induced dilation of large epicardial coronary arteries in conscious dogs.[17] However, in the context of our experimental conditions, it is important to consider that NO has been shown to prevent EDHF production.[18,19] Thus, despite the fact that numeric addition of the NO-dependent (≈30%) and EDHF-dependent (≈60%) responses corresponds to the relaxation mediated by substance P (≈90%), the contribution of EDHF to the combined response cannot be ascertained.

Endothelium-dependent relaxation was reduced by the different in vivo interventions used in this study (Figure 5). Immediately, ICRT or PTCA alone did not affect NO-dependent relaxation, but it was reduced when these 2 procedures were combined. After 6 weeks, ICRT alone reduced substance P–mediated NO-dependent relaxation of the vessels. In combination with PTCA, this response was absent. Thus ICRT damaged the endothelium-dependent release or availability of NO, which was exacerbated when combined with PTCA.

EDHF-dependent relaxation mediated by substance P was decreased immediately after ICRT and PTCA, individually and in combination. This relaxation partly recovered 6 weeks after ICRT but not after PTCA and combined PTCA+ICRT.

Several reasons may explain this result. There are functional signs of regeneration of the endothelium 6 weeks after PTCA and ICRT alone that are clearly absent after the double procedure. Second, even if physical endothelial regeneration is complete, it has been shown that the new endothelium may be functionally deficient.[20] Surprisingly, however, whereas substance P–induced relaxation was trivial 6 weeks after PTCA+ICRT, removal of the endothelium potentiated the contraction induced by $PGF_{2\alpha}$. This implies that receptor-operated release of EDRFs is absent 6 weeks after PTCA+ICRT, but the endothelium still prevents contraction and thus constitutively produces EDRFs.

Removal of the endothelium potentiated the relaxant effect of SNP in control arteries (Figure 6), reflecting an increased guanylate cyclase sensitivity associated with the loss of NO.[21] This supersensitivity to nitrovasodilators has also been described in vivo after inhibition of vascular NO synthase.[22]

The potentiating effect of endothelial removal on SNP-induced relaxation was blunted in all groups, suggesting a lack of endothelium-dependent regulation of smooth muscle sensitivity to SNP. The origin of this alteration is unknown because it is not specific for 1 type of procedure (ICRT or PTCA). The only link between these changes is the endothelial dysfunction, since in denuded vessels SNP-induced relaxation is similar in all groups except immediately after PTCA. In this latter case, it is possible that rubbing the endothelium immediately after the PTCA but not after healing damaged the smooth muscle, as shown by the decrease in sensitivity to SNP. Also, a clear increase in SNP-induced relaxation was observed after long-term PTCA+ICRT. This is most likely to be associated with the decreased NO production and/or release observed under stimulated conditions (Figure 5). This would be in agreement with previously published data showing that NO synthase inhibition increases guanylate cyclase sensitivity both in vitro and in vivo.[21,22]

It is important to note that our results differ from a recent in vivo study[6] in which SNP-induced dilation was absent immediately and in the long term after ICRT, whereas acetylcholine-induced relaxation was restored after 5 weeks. However, we have no explanation for this discrepancy other than the different methodological approach.

The identical response of the 2 groups of controls from irradiated and nonirradiated animals suggests that the effects are entirely local and that a significant systemic or distant effect, as has been previously suggested,[5] can be excluded.

Clinical Implications

The implications of endothelial dysfunction over a short vessel segment are unclear. First, a patent arterial segment that has impaired reactivity is a scenario not dissimilar to a stented segment. This is clearly preferable to a restenosed segment. This may have different implications if the treated segment is long. Second, the reduction of vessel wall integrity, as shown by a reduction in baseline tension, mandates longer-term follow-up than usual in the clinical situation. Angiographic and intravascular ultrasound studies can provide bountiful information regarding the stability of these vessel segments.[4] Finally, because of the severity of endothelial dysfunction demonstrated, the possibility of very delayed regeneration must be entertained. Although this study was performed on normal porcine arterial rings, its findings have significant implications for a treatment currently under extensive clinical investigation.

Limitation of the Study

Because this study was performed immediately and at 6 weeks after intervention, no conclusion can be drawn regarding the progression of this process. Furthermore, the effects demonstrated are limited to the site of the intervention and the response of proximal and distal vessel segments were not assessed, although the reactivity of nontreated coronary arteries from animals in which another coronary vessel had been irradiated was unaltered. Finally, atherosclerosis being the background of this clinical intervention, we do not know what the impact would be of PTCA+ICRT in this context. This study was designed to investigate the effects of PTCA+ICRT and ICRT alone on coronary reactivity and endothelial function; our data cannot predict the outcome of PTCA+ICRT in a hypercholesterolemic/atherosclerotic clinical background.

Conclusions

PTCA+ICRT altered the passive mechanical properties of porcine coronary arterial wall. Furthermore, receptor-operated release of endothelium-derived NO and EDHF were reduced by ICRT and PTCA alone and prevented by the combination of PTCA+ICRT. The ongoing impairment of endothelial function after 6 weeks in this model further supports the longer-term follow-up of all patients undergoing post-PTCA irradiation.

Acknowledgments

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