Comparison of Proliferative Activity in Coronary Plaques from Patients with Coronary Ischemia
Histopathological and Immunohistochemical Analysis

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ABSTRACT

The overgrowth of cells of the vessel wall, especially of the smooth muscle cells (SMCs), contributes to the pathogenesis of coronary atherosclerosis and wound repair after coronary angioplasty. However, the association between cellular proliferation in coronary lesions and clinical pathophysiology remains to be clarified in humans. Thus, we investigated proliferative activity in coronary tissues obtained from patients with coronary ischemia. The proliferative activity in tissues obtained by using directional coronary atherectomy (DCA) from 87 coronary lesions was assessed by immunohistochemical staining for the proliferating cell nuclear antigen (PCNA). The lesions were divided into 34 primary lesions and 53 postangioplasty lesions. The 34 primary tissue samples were obtained from 9 patients with stable angina pectoris (SAP) and 25 patients with acute coronary syndromes (ACS). Collectively, the 53 postangioplasty tissue samples were obtained from 37 patients with SAP and 16 patients with ACS. The PCNA labeling index (LI) was quantified as the mean percentage of PCNA-positive cells in the 3 most positive high-power fields ($\times 200$). The mean LIs were high in the primary ACS samples [8.9 ± 2.1% (p=0.01)] and postangioplasty samples [2.3 ± 0.8% (p=0.08) in SAP cases and 4.1 ± 2.4% (p=0.06) in ACS cases] compared with the primary SAP samples (0.2 ± 0.2%). Intimal hyperplasia, a random proliferation of SMCs (α-actin positive) was marked in the primary ACS samples (76%) as well as in the postangioplasty SAP (92%) and ACS (81%) samples, as compared with the primary SAP samples (33%) (p<0.01). PCNA expression was mainly evident in the nucleus of the SMCs and CD68-positive macrophages. Many PCNA-positive cells were localized in plaque areas, as follows: intimal hyperplasia, neovascularized lesions, lesions with macrophage clusters, and lesions near areas of disrupted internal elastic lamina. The levels of PCNA expression in coronary lesions were not associated with the subsequent development of restenosis after DCA. Our findings suggest that the excessive proliferation of vascular wall cells, especially SMCs, is involved in the pathogenesis of ACS and in the process of wound repair after angioplasty in humans.

Key words: Proliferating cell nuclear antigen (PCNA), Directional coronary atherectomy (DCA), Acute coronary syndromes, Restenosis

The onset of acute coronary syndromes (ACS: unstable angina, acute myocardial infarction, and sudden cardiac death) as well as restenosis after percutaneous transluminal coronary angioplasty (PTCA), which occurs in 30% to 50%[18,27,42] of angioplasties, present clinical problems. Elucidation of the pathogenesis of coronary plaque formation and the process of restenosis would help to refine treatment. The overgrowth of the cells of the vessel wall, especially the smooth muscle cells (SMCs), is believed to be important in the pathogenesis of coronary atherosclerosis[3,12,38] as well as in restenosis after coronary angioplasty[50,53]. Indeed, intimal hyperplasia, a manifestation of SMC proliferation, has been demonstrated in coronary atherosclerosis and restenotic lesions[22,41].

Proliferating cell nuclear antigen (PCNA) is a

36 kDa acidic non-histone nuclear protein that is used as a proliferation marker. This molecule functions as an auxiliary protein for DNA polymerase δ and is an absolute requirement for DNA synthesis[6,16,33]. Central to PCNA utility is its uniform expression during the late G1, S, and G2 phases of the cell cycle[5,20]. Expression of PCNA correlates well with other indexes of proliferation, such as ^{3}H-thymidine, Ki-67, and 5-bromodeoxyuridine (BrdU) labeling[21,55]. More et al[23,24] reported that the timing and localization of cellular proliferation in vascular walls can be successfully monitored by using a monoclonal antibody against PCNA in a rabbit angioplasty model. Recent studies using directional coronary atherectomy (DCA) tissues also reported good immunohistochemical assessment of PCNA expression in human coronary lesions[29,32]. However, the results of these DCA studies were controversial in frequencies and levels of cellular replication. Moreover, the association between cellular proliferation in coronary lesions and clinical pathophysiology has not been thoroughly delineated.

Therefore, we investigated the level of cellular proliferative activity in coronary tissues obtained by DCA from patients with coronary ischemia.

MATERIALS AND METHODS

Human coronary atherectomy tissue

We analyzed coronary tissues obtained from 87 coronary lesions of 87 consecutive patients with coronary ischemia who underwent DCA between May 1993 and May 1995. Previously described DCA techniques[17,45] were used in patients who demonstrated a significant coronary narrowing and a favorable anatomy of the lesion[16] after informed consent had been obtained.

The lesions examined were divided into 34 primary lesions with no history of coronary angioplasty and 53 postangioplasty lesions (post PTCA, n=41; post DCA, n=12), on which previous coronary angioplasties had been performed. The 34 primary tissue samples were obtained from 9 patients with stable angina pectoris (SAP) and 25 patients with ACS. These ACS patients included 19 cases of unstable angina pectoris (UAP), as defined by Braunwald[4] (class IB, n=10; class IIB, n=8; class IIIB, n=1), and 6 cases of acute myocardial infarction (AMI). Collectively, the 53 postangioplasty tissue samples were obtained from 37 patients with SAP and 16 patients with ACS.

Following DCA, the atherectomy specimens were immediately immersed in fresh, ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, for 2 to 4 hours. The samples were then transferred to a sterile 30% sucrose/PBS solution and stored overnight at 4°C. All sample tissues, embedded separately in O.C.T. compound (Tissue-Tek®, Miles, Inc., Elkhart, IN, USA) into a few tissue blocks, were snap frozen in liquid nitrogen and stored at –80°C until use. We investigated these tissue blocks to make histopathological and immunohistochemical evaluation. Frozen sections (5 μm) were cut on a cryostat, placed on slides coated with 3-aminopropyl-trimethoxysilan, and stored in sealed boxes at –80°C until assayed. One section from each specimen was stained with hematoxylin and eosin for conventional light microscopic analysis, and the remaining sections were used for immunohistochemical evaluation.

Antibodies

The primary antibodies employed in this study were HHF35 (anti-α-smooth muscle actin, Dako, Corp., Carpinteria, CA, USA, dilution 1:30) for the identification of SMCs; anti-CD68 (KP1, Dako, dilution 1:80) or HAM56 (Dako, dilution 1:50) for monocytes/macrophages; anti-CD45 (T29/33, Dako, dilution 1:80) for lymphocytes, and anti-CD31 (JC/70A, Dako, dilution 1:30) for the identification of endothelial cells. Anti-proliferating cell nuclear antigen (PCNA) (PC10, Dako, dilution 1:50) was used for evaluation of proliferative activity, as previously described[14,29,32,48]. For all immunohistochemical analyses, the HHF35 and PC10 primary antibodies were incubated with their respective tissue sections for 2 h at room temperature (RT). The remaining primary antibodies were incubated overnight at 4°C.

Immunohistochemistry

The frozen sections were washed in PBS containing 0.1% Triton X-100 for 5 min, followed by 5 × 2 min washes in PBS at 4°C. Endogenous peroxidase activity was blocked by incubating the samples with 0.3% hydrogen peroxide (H_2O_2) in methanol at RT for 20 min, followed by immersion into 70% ethanol for 1 min. After 3 additional washings in PBS containing 0.1% Triton X-100 at 4°C, normal horse (HHF35 antibody, CD68 antibody, CD45 antibody, PC10 antibody) or goat (HAM56 antibody) serum was dripped onto the slide for 20 min at RT in order to block nonspecific reactivity, prior to incubation with the primary antibodies. As negative controls, normal serum (mouse or goat), diluted with an equal volume of PBS, was substituted for the primary antibodies. All treated sections were then incubated with biotinylated secondary antibody at RT, followed by incubation with an avidin-biotinylated horseradish peroxidase complex (Avidin: Biotinylated enzyme Complex method, Vectastain® Elite ABC kit, Vector Laboratories, Inc, Burlingame, CA, USA). The peroxidase reaction was visualized by applying 20 mg of 3, 3'-diaminobenzidine tetrahydrochloride (DAB) and 20 μl of 30% H_2O_2 diluted in 100 ml of 0.05 M tris buffer, pH 7.6, for 2 to 3 min, followed by a 25 min immersion in 100 ml

of 0.05 M tris buffer, pH 7.6, containing 1 ml of dimethyl sulfoxide and 20 mg of DAB at RT. The sections were subsequently counterstained with methyl green.

Histochemical and immunohistochemical analyses

All specimens were evaluated by light microscopy for the presence of coronary plaque components, which were classified as dense fibrous tissue, intimal hyperplasia, pultaceous debris, thrombus, calcification, neovascularization, intraplaque hemorrhage, or inflammatory infiltrates (macrophages and lymphocytes).

Dense fibrous tissue (hard, collagen-rich sclerotic tissue) was seen as an area of relatively acellular tissue composed primarily of dense collagen fibers[19]. Intimal hyperplasia, seen as fibromuscular connective tissue, was characterized by the random proliferation of stellate to spindle-shaped SMCs (a-actin positive) in a loose myxoid stroma[8,13,41]. Pultaceous debris (soft, lipid-rich atheromatous gruel) constituted pale-staining areas that were co-mixed with necrotic debris, amorphous extracellular matrix, cholesterol clefts, and foam cells. Thrombus was considered to be present whether recent or more organized in appearance, as previously described[37]. Calcification was detected by dark purple granular staining areas[19]. Neovascularization was considered to be present if more than three microvessels with tube formation were detected in a high-power field ($\times 200$). Intraplaque hemorrhage included both bleeding and hemosiderin deposition, the evidence of a previous hemorrhage. Macrophage (CD68 or HAM56 positive cell) and lymphocyte (CD45 positive cell) infiltrates were considered to be present when these cells formed clusters, i.e., if more than thirty immunopositive cells were detected in a high-power field ($\times 200$).

Proliferative activity was assessed by the level of PCNA expression. The nuclear labeling index for PCNA was quantified as the percentage of cells having the entire nucleus labeled dark-brown, as assessed in the 3 most PCNA-positive cellular high-power microscope fields ($\times 200$). We considered the nuclei to be positive or negative without regard for PCNA staining intensity.

Angiographic follow-up

Coronary angiograms were obtained before and immediately after atherectomy and at follow-up by cardiologists with no knowledge of the histopathological and immunohistochemical findings. To assess lesion severity, the lesions were measured with a cardiac image analysis system (POLYC2 LARC2 DCI, Coronary Quantification Software Package, Philips Medical Systems, The Netherlands). A follow-up angiographic study was recommended more than three months after

DCA, but was performed earlier if clinically indicated. The amount of acute gain was defined as the percent difference in the diameter of the lesion before and after atherectomy. A successful DCA was defined as a residual diameter stenosis of <50%. Restenosis was defined as the presence of stenosis ≥50% at the atherectomy site at follow-up.

Statistical analyses

Data are expressed as a percent or mean ± SD. Immunohistopathological results are expressed as a percent. Differences between data sets were compared by one-way ANOVA for continuous variables, and by multiple-comparison contingency table analysis with the chi-squared test or Fisher's exact probability test for dichotomous variables. The PCNA labeling index is expressed as mean ± SEM. Linear regression analysis (Pearson's correction) was used to define correlations between different parameters. When comparison of the PCNA labeling indices for the four clinical groups by the Kruskal-Wallis test demonstrated statistical significance, the Mann-Whitney U-test with Bonferroni's correction was applied for comparing the two groups. Differences were considered significant at a value of $p<0.05$.

RESULTS

The clinical characteristics of the patients recruited for this study are presented in Table 1. There were 78 men and 9 women, mean age 62.1 ± 10.6 years (range 32 to 88 yrs). Mean age was low in the patients with ACS among the postangioplasty cases, as compared with the other patient groups ($p=0.04$). The other baseline characteristics were similar in the four groups.

Coronary plaque component

The prevalence of coronary plaque components is summarized in Table 2. Intimal hyperplasia was marked in the primary tissue samples obtained from ACS patients (76%) as well as in the postangioplasty tissue samples from SAP (92%) and ACS (81%) patients, whereas in the primary tissue samples obtained from patients with SAP, hyperplasia was minimal (33%) ($p<0.01$). The prevalence of intraplaque hemorrhage and inflammatory infiltrates (macrophages and lymphocytes) was high in the primary tissue samples obtained from patients with ACS, as compared with the postangioplasty tissue samples and primary tissue samples obtained from patients with SAP ($p<0.05$). There was a trend toward an increased prevalence of neovascularization among the primary ACS patients compared with the other patient groups ($p=0.09$). However, this did not achieve statistical significance. The prevalence of the remaining components did not differ significantly among the patient groups. The typical

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Table 1. Clinical characteristics

	Primary		Postangioplasty		p
	SAP (n=9)	ACS (n=25)	SAP (n=37)	ACS (n=16)	
Clinical data					
Male sex, n (%)	9 (100)	23 (92)	31 (84)	15 (94)	NS
Age (years)	63.3 ± 14.9	62.3 ± 10.2	64.4 ± 8.9	55.4 ± 10.1	0.04
BMI (kg/m²)	22.8 ± 3.5	23.4 ± 2.2	24.2 ± 3.2	23.5 ± 3.6	NS
Systemic hypertension, n (%)	7 (78)	10 (40)	22 (59)	7 (44)	NS
Diabetes mellitus, n (%)	4 (44)	5 (20)	15 (41)	8 (50)	NS
Cigarette smoking, n (%)	5 (56)	12 (48)	16 (43)	11 (69)	NS
Medications					
Calcium blockers, n (%)	9 (100)	23 (92)	30 (81)	14 (88)	NS
Nitrates, n (%)	9 (100)	23 (92)	30 (81)	14 (88)	NS
Antiplatelet agents, n (%)	8 (89)	25 (100)	35 (95)	16 (100)	NS
ACE inhibitors, n (%)	2 (22)	5 (20)	8 (22)	2 (13)	NS
Angiographic data					
Pre-DCA% stenosis	73.9 ± 5.9	74.3 ± 15.2	73.7 ± 12.2	76.3 ± 16.8	NS
Post-DCA% stenosis	22.5 ± 17.1	13.7 ± 13.5	20.9 ± 13.3	14.9 ± 16.3	NS

SAP: stable angina pectoris; ACS: acute coronary syndromes; BMI: body mass index; ACE: angiotensin converting enzyme; DCA: directional coronary atherectomy; NS: not significant.

Table 2. Histopathological and immunohistochemical characteristics

	Primary		Postangioplasty		p
Components, n (%)	SAP (n=9)	ACS (n=25)	SAP (n=37)	ACS (n=16)	
Dense fibrous tissue	9 (100)	23 (92)	34 (84)	12 (75)	NS
Intimal hyperplasia	3 (33)	19 (76)	34 (92)	13 (81)	<0.01
Pultaceous debris	2 (22)	8 (32)	13 (35)	7 (44)	NS
Thrombus	4 (44)	18 (72)	20 (54)	11 (69)	NS
Calcification	5 (56)	17 (68)	22 (60)	6 (38)	NS
Neovascularization	1 (11)	14 (56)	14 (38)	7 (44)	0.09
Hemorrhage	2 (22)	19 (76)	13 (35)	4 (25)	<0.01
Macrophages	3 (33)	20 (80)	18 (49)	9 (56)	<0.05
Lymphocytes	2 (22)	18 (72)	10 (27)	7 (44)	<0.01

SAP: stable angina pectoris; ACS: acute coronary syndromes; NS: not significant.

components of the coronary lesions are shown in Fig. 1.

Proliferative activity in human coronary lesions

SMCs (α-actin positive) (Fig. 2, panels b and c) and macrophages (CD68 positive) (Fig. 2, panels e and f) were the predominant proliferating cell types found in the coronary lesions. Large numbers of PCNA-positive cells were localized in plaque areas, as follows: intimal hyperplasia (Fig. 2c), lesions with clustered macrophages (Fig. 2f), neovascularized lesions (Fig. 3), and lesions near areas of disrupted internal elastic lamina (Fig. 4).

Fifty-three percent (46/87) of all atherectomy samples were PCNA-positive. The occurrence of PCNA expression in the primary ACS samples (64%, 16/25) and in the postangioplasty SAP (51%, 19/37) and ACS (56%, 9/16) samples was higher (p=0.2) than in the primary SAP samples (22%, 2/9). However this did not achieve statistical significance. The PCNA labeling index averaged 4.3 ± 0.9% (range 0 to 35.7%). The mean PCNA labeling indices were also high in the primary ACS samples [8.9 ± 2.1% (p=0.01)] and postangioplasty samples [2.3 ± 0.8% (p=0.08) in SAP cases and 4.1 ± 2.4% (p=0.06) in ACS cases] compared with the primary SAP samples (0.2 ± 0.2%) (Fig. 5). In the postangioplasty tissues there was no difference in the labeling index between the patients with SAP (n=37) and those with ACS (n=16), (p=0.83).
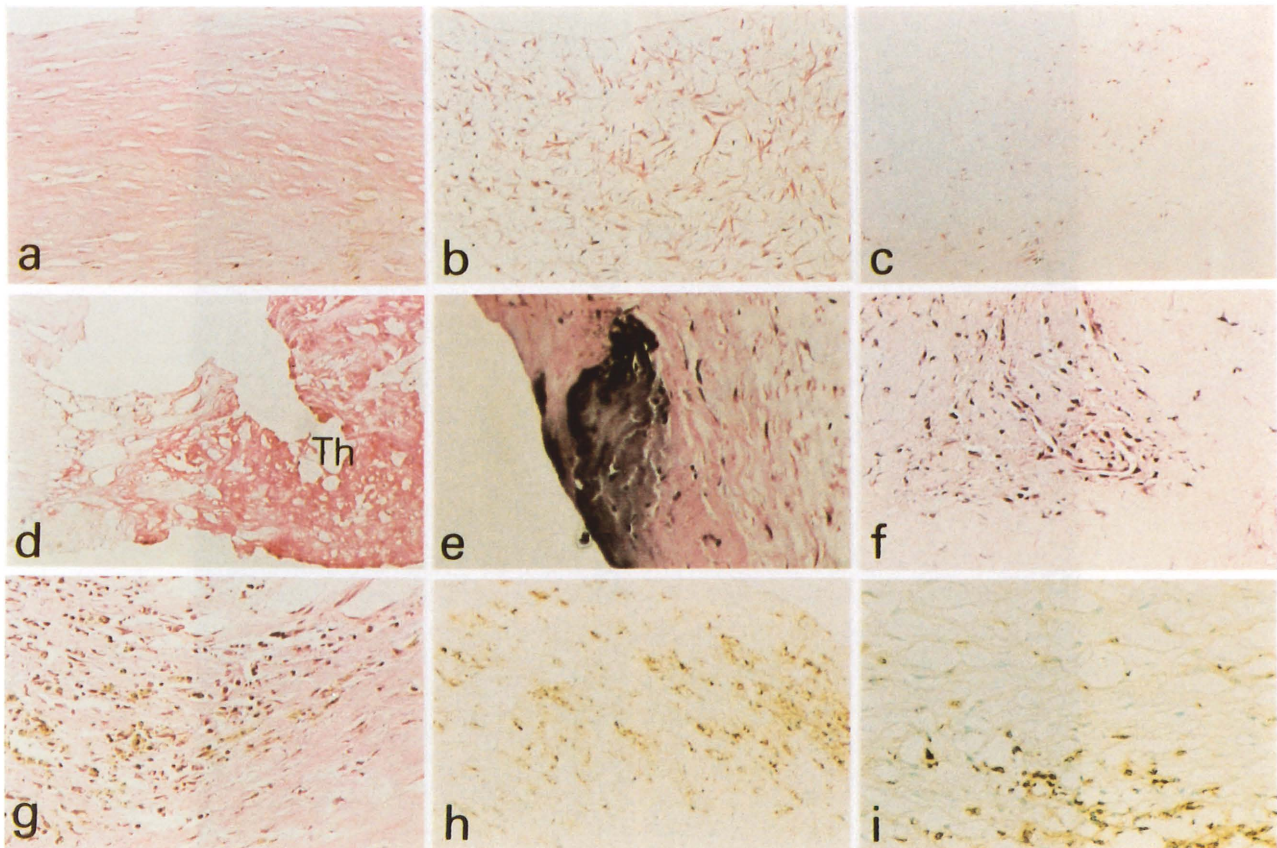
Fig. 1. Typical plaque components in vessels from patients with acute coronary syndromes.
Panel a, dense fibrous tissue; panel b, intimal hyperplasia characterized by random proliferation of stellate to spindle-shaped cells in a loose myxoid stroma; panel c, pultaceous debris; panel d, thrombus (Th) attached to pultaceous debris; panel e, calcification (dark purple stain); panel f, neovascularization (tube formation); panel g, hemosiderin deposition with inflammatory infiltrates; panel h, clusters formed by macrophages; and panel i, clusters formed by lymphocytes. Panels a through g are stained with hematoxylin-eosin (HE); panel h is immunostained with anti-human macrophage (CD68); and panel i is immunostained with anti-human lymphocyte (CD 45). Original magnification, a through d, ×100; e through g, ×200; h, ×100; i, ×200.

The PCNA labeling index was not correlated with any angiographically visualized stenosis in either the primary cases (Fig. 6) or postangioplasty cases (data not shown). Nor was there any significant relationship between the age of the patient and the PCNA labeling index. Other clinical variables (body mass index, diabetes mellitus, smoking, hypertension, and drug medication) were also not associated with the level of expression of PCNA.

In the primary tissue samples obtained from patients with ACS, the PCNA labeling index was higher, but not to a significant extent, in the patients with UAP (10.5 ± 2.6%, n=19) compared with those with AMI (3.7 ± 2.9%, n=6), (p=0.09).

In the postangioplasty tissue samples, the degree of proliferation was most evident (35.7%) in one patient with acute occlusion one day after PTCA. However, there was no significant correlation between the time interval (<1 year) after a previous angioplasty and the PCNA labeling in-

dex [r=−0.06 (n=35, p=0.71) in SAP cases and r=−0.27 (n=13, p=0.38) in ACS cases] (Fig. 7). Proliferating cells were seen at all times throughout the first year after angioplasty. There was no difference in the labeling index between the post-PTCA lesions (n=41) and the post-DCA lesions (n=12) (2.6 ± 1.0% vs. 3.8 ± 2.0%, p=0.22).

Follow-up angiography

There were three lesions with a residual diameter stenosis of ≥50%. At the early follow-up (<3 months), 3 patients had experienced acute coronary events with restenosis detected angiographically. Angiography for a late follow up study (≥3 months) was performed in 61 patients. One patient with acute occlusion after DCA and 19 patients without late follow up (≥3 months) catheterization could not be enrolled in the follow-up study of subsequent restenosis after DCA. Follow-up angiographic findings were obtained for a total 64 lesions. These lesions were divided
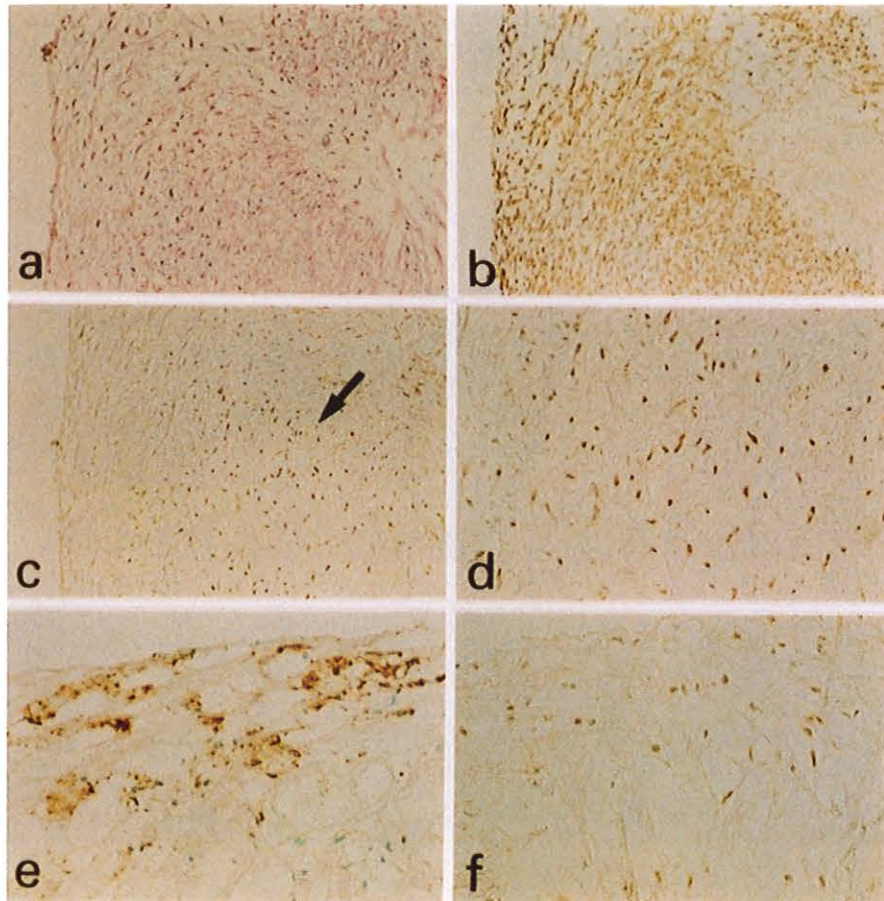
Fig. 2. Immunolabeling of serial sections for proliferative cell nuclear antigen (PCNA) in SMCs (panels a through d) and macrophages (panels e through f).
Panel a, hematoxylin-eosin staining; and panel b immunostaining for smooth muscle cells (α-actin). Panels c and d, immunostaining for PCNA. Brown nuclei indicate proliferation. Panel d is a high-power magnification of panel c (arrow head). Panel e, immunostaining for macrophages (HAM56). Panel f, immunostaining for PCNA. Original magnification, a through c, $\times 100$; d, $\times 200$; e and f, $\times 200$.

into two groups: a no PCNA expression group (group N, n=41); and a PCNA expression group (group P, n=46). Furthermore, since the median of the PCNA labeling index in the PCNA expression cases was 3.3%, group P was divided into two groups, as follows: group L, PCNA labeling index 0 to 3.3%, n=23; and group H, PCNA labeling index $\geq 3.3\%$, n=23. There were no differences in the baseline clinical characteristics and follow-up rates (group N, 76%; group L, 87%; group H, 57%) among the three degrees of PCNA expression. The degree of stenosis and the incidence of restenosis at follow up did not differ among the three groups (Table 3). Similarly, in the primary lesions, there was no relationship between the presence of PCNA expression and subsequent restenosis after DCA. This suggests that the level of PCNA expression in coronary lesions does not influence the subsequent development of restenosis after DCA.

DISCUSSION

This study demonstrated that the level of vascular cell proliferation paralleled the level of expression of PCNA in coronary tissues obtained from patients with SAP or ACS (unstable angina and myocardial infarction) and in postangioplasty tissues. Intimal hyperplasia, the random proliferation of SMCs, was highly evident in both primary tissue samples obtained from ACS patients and postangioplasty tissue samples compared with primary tissue samples from SAP patients. In addition, the levels of proliferative activity in the primary ACS and postangioplasty samples were higher than in the primary SAP samples, suggesting that cellular proliferation, especially the proliferation of SMCs, is one of the mechanisms in the development of ACS and wound repair following angioplasty in humans.

The sudden transformation of a stable angina to life-threatening ACS[9,10,19,36,51] is supported by the evidence of plaque rupture leading to
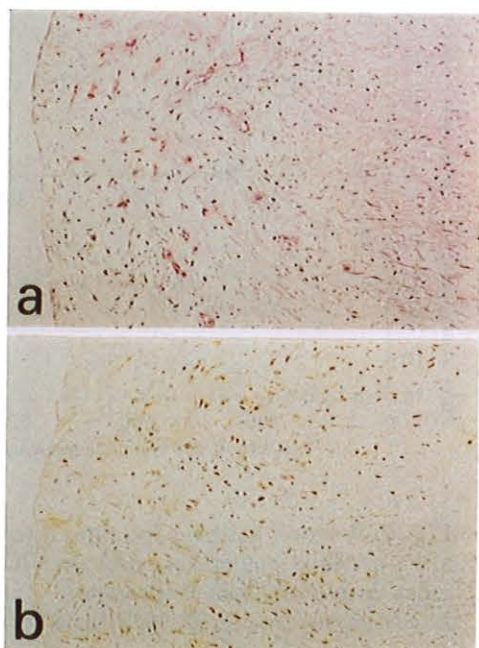
Fig. 3. PCNA-positive cells in neovascularized lesions.
Panel a, neovascularized tissue with tube formation (hematoxylin-eosin staining). Panel b, immunostaining for PCNA. Original magnification, ×100.
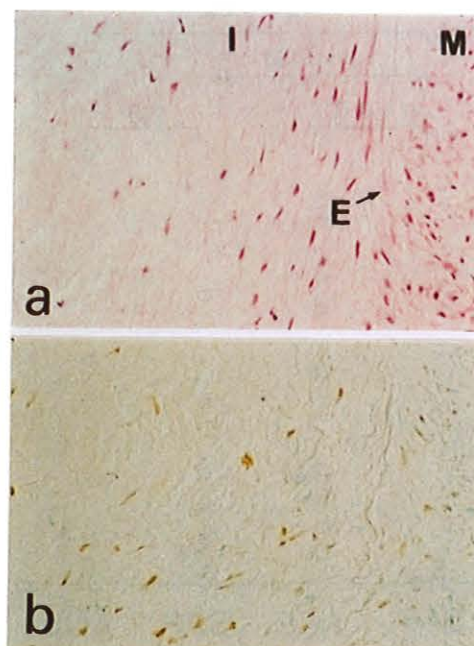
Fig. 4. Immunoreactivity for PCNA in areas of disrupted internal elastic lamina. Panel a, the intima-media lesion (I, intima; E, internal elastic lamina; M, media), stained with hematoxylin-eosin. Panel b, immunostaining for PCNA. Original magnification, ×200.

thrombosis, while postangioplasty restenosis[28,49,50,53] is due largely to neointimal hyperplasia caused by excessive SMC proliferation. The incidence of intimal hyperplasia in postangioplasty cases was marked in our series, confirming previously reported observations showing that intimal hyperplasia is the representative morphology for restenosis after coronary angioplasty[13,40]. Intimal hyperplasia induced by injury reportedly results from abnormal wound healing characterized as an excessive inflammatory response of the vascular wall marked by SMC proliferation and the production of loose connective tissue[13,39]. In agreement with a previous study[41], we observed intimal hyperplasia in numerous primary lesions. Escaned et al[8] reported that intimal hyperplasia represented an unspecific vessel wall response to different types of injury including shear stress and virus-related endothelial injury. Rosenschein et al[37] reported an increase of intimal hyperplasia in tissues obtained by DCA from patients with ACS. Lipid-rich pultaceous debris, a more dangerous component that contributes to plaque rupture and thrombosis, has also been found at high levels in lesions responsible for ACS[7–9,37]. However, our results showed the incidence of pultaceous debris and thrombi in our cases of ACS to be no higher than that in the other patient groups. This discrepancy may be explained by the effect of sampling error, including the loss of lip-
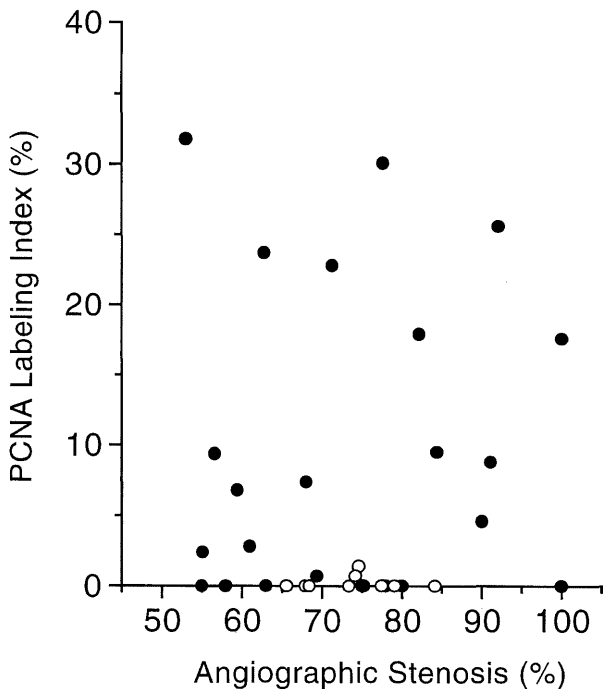
ids from the tissue specimen during processing, degradation of thrombus caused by spontaneous lysis or by the concomitant administration of intravenous heparin, or a superficial thrombus being pushed downstream when the atherectomy device crosses the lesion, as previously reported[8,37].

The present study demonstrated that neovascularization, intraplaque hemorrhage, and inflammation were more evident in primary ACS cases as compared with the other groups of patients. In this regard, the rupture of the coronary vasa vasorum may be involved in the onset or triggering of a myocardial infarction[2]. Patterson speculated that repeated intimal hemorrhage eventually leads to coronary artery thrombosis[31]. Our findings corroborate these previous reports. Evaluation of atherectomy specimens obtained from patients with ACS revealed areas significantly rich in macrophages, as compared with specimens obtained from patients with SAP[25]. Van der Wal et al[51] reported the presence of numerous inflammatory cells at the site of plaque rupture. These findings, along with ours, suggest that the risk of plaque disruption is an ongoing inflammatory process within the macrophage infiltrate.
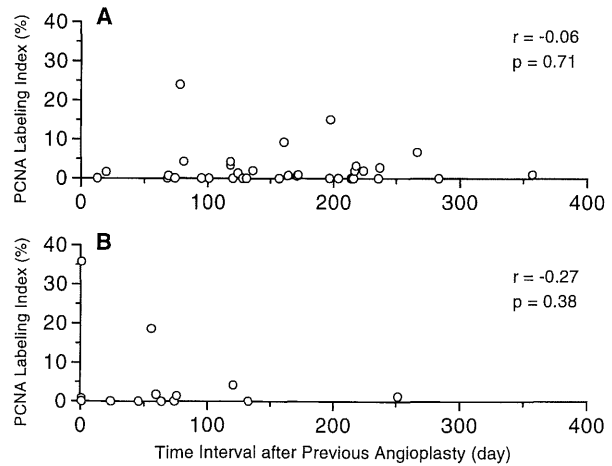
PCNA immunoreactivity was mainly observed in the SMCs and macrophages in the present study. A previous study[29] using double-labeling
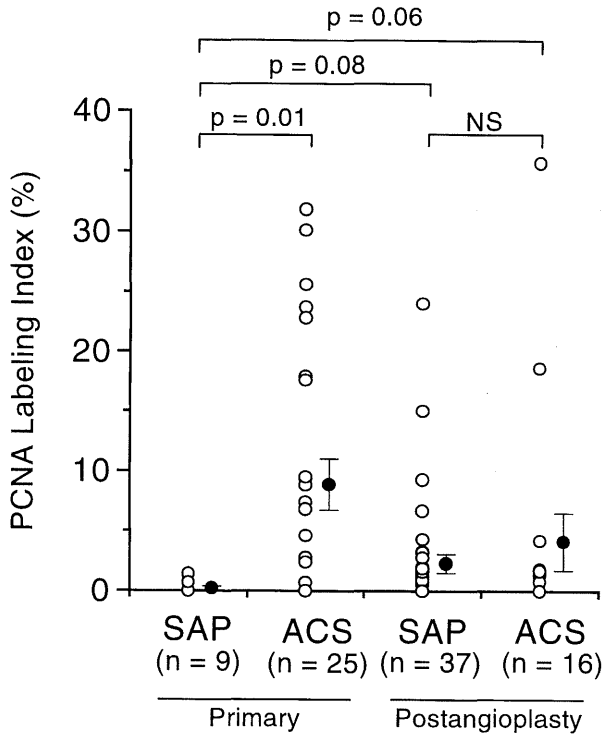
Fig. 5. PCNA labeling index (LI) in tissue samples obtained from patients with coronary ischemia.
Statistical analysis by Kruskal-Wallis test revealed a significant difference between the four clinical groups (p=0.02). SAP: stable angina pectoris; ACS: acute coronary syndromes. (○)=individual LI. (●)=mean LI. Values are mean ± SEM.

Fig. 7. Relation between the time interval (<1year) after previous angioplasty and the PCNA labeling index in postangioplasty tissues.
A: SAP cases (n=35), B: ACS cases (n=13).

Fig. 6. Correlation between angiographically visualized stenosis and the PCNA labeling index in primary tissue samples.
○: SAP cases (r=0.053, p=0.89, n=9); ●: ACS cases (r=0.01, p=0.96, n=25).

immunohistochemistry reported that the mean percentage of each PCNA-positive cell type was 62.7% vs. 63.5% in the SMCs, 32.5% vs. 24.9% in the macrophages, and 11.5% vs. 14.6% in the endothelial cells (primary specimens vs. restenotic specimens). Rekhter et al[35] also reported that the main proliferative cell type in the intima was the monocyte/macrophage and in the media, the SMCs. In the present study, marked immunoreactivity for PCNA was evident in the areas of plaque associated with intimal hyperplasia, neovascularized lesions, or lesions with clusters of macrophages. O'Brien et al[29,30] reported microvessel-associated cell proliferation in the coronary lesions of patients. While the trigger for such an increase in cell replication is unclear, the process is likely mediated via the expression of growth factors, particularly because previous studies[34,54] have demonstrated the co-localization of platelet-derived growth factor (PDGF) with plaque microvessels. Arbustini et al[1] have shown elevated levels of basic fibroblast growth factor (bFGF) and PDGF AA-positive cells in coronary tissues with neointmal hyperplasia. Our preliminary data also show that the level of expression of transforming growth factor beta (TGF-β), a growth regulatory factor, was significantly increased in areas of intimal hyperplasia and neovascularization in human coronary tissues (unpublished observation). In addition, a marked proliferation of cells was observed in the lesions near areas of disrupted internal elastic lamina. This finding may imply that the migration of proliferating SMCs from the media to the intima contributes to furthering intimal hyperplasia. Some animal models of balloon arterial injury demonstrated PCNA-positive cells in the media soon after injury, prior to their migrating into the

Table 3. Proliferative activity and angiographic findings in coronary lesions

		Group N (n=41)	Group L (n=23)	Group H (n=23)	p
RCA	n (%)	15 (37)	4 (17)	7 (30)	NS
LAD	n (%)	25 (61)	17 (74)	16 (70)	NS
LCx	n (%)	1 (2)	2 (9)	0 (0)	NS
Pre-DCA stenosis	(%)	73.8 ± 11.8	72.4 ± 12.2	77.2 ± 17.0	NS
Post-DCA stenosis	(%)	18.2 ± 13.7	18.0 ± 13.6	17.3 ± 17.4	NS
Acute gain	(%)	55.6 ± 19.7	54.4 ± 18.1	60.0 ± 18.0	NS
Follow-up					
Follow-up rate	n (%)	31 (76)	20 (87)	13 (57)	NS
Follow-up stenosis	(%)	53.6 ± 28.9	52.4 ± 28.2	45.7 ± 28.5	NS
Restenosis rate	n (%)	17 (55)	10 (50)	6 (46)	NS

Group N: no expression of PCNA; Group L: PCNA labeling index 0 to 3.3%;
Group H: PCNA labeling index ≥ 3.3%; NS: not significant.
RCA: right coronary artery; LAD: left anterior decending coronary artery; LCx: left circumflex artery. Restenosis was defined as more than 50% stenosis upon follow-up angiography.

intima[23,47,57].

The amount of PCNA expression in human atherosclerotic lesions is controversial. Previous studies[29,48] have demonstrated low levels of PCNA expression, with the majority reporting less than 1% positive cells per slide, and there were no differences in PCNA expression levels between primary and restenotic lesions. In contrast, Pickering et al[32] demonstrated that all primary and restenotic tissue specimens obtained from active coronary and peripheral lesions had much higher percentages (mean, 15.2%) of positive cells as determined by immunohistochemical methods, and that the percentage of PCNA-positive cells was higher in the restenotic than primary lesions. The sensitivity of such methods can be influenced by factors including the duration and type of fixation, the method of tissue processing, the nature, density or location (nuclear, cytoplasmic, membrane related or extracellular) of the antigen in the tissue specimen, the antibodies and labeling systems used, and the experience of the laboratory personnel. The present results, which used frozen sections, can not be compared with the results of previous studies[29,32,48] that used paraffin sections. Accordingly, an important implication of PCNA expression is not the overall amount of expression in individual tissue but rather its differential expression among various clinical groups when the same antibodies and immunohistochemical methods are used and the same evaluation is performed.

We found PCNA expression to be most evident in primary tissues obtained from patients with ACS, i.e., those with acute ischemic symptoms. Pickering et al[32] indicated that in certain primary plaques with numerous (>10%) proliferating cells, the rapid growth of some primary atherosclerotic lesions may involve localized increased SMC proliferation. It has been suggested that, in a subset of patients with unstable angina, an accelerated type of intimal hyperplasia may lead to partial vessel obstruction without evidence of plaque disruption or thrombosis[1,12]. Moreover, Flugelman et al[12] found that unstable angina lesions displayed high levels of expression of acidic and basic FGFs. Our findings of an increase in proliferative activity and a prevalence of intimal hyperplasia in primary ACS cases (76%) may support this mechanism of unstable angina.

Despite the evidence of intimal hyperplasia (92% in SAP cases and 81% in ACS cases) in postangioplasty tissues compared with that in primary tissues obtained from SAP patients, the expression of PCNA in the postangioplasty samples was not significantly higher than that in the primary SAP samples. This discrepancy could result from an early burst of SMC proliferation after angioplasty and later atherectomy tissue sampling, as mentioned previously[29,48]. In fact, in our study, a case of acute occlusion one day after PTCA demonstrated the highest proliferation level (35.7%), while the majority of postangioplasty coronary tissues were obtained more than two weeks after previous coronary angioplasty. Recently, antisense PCNA oligonucleotides have been reported to inhibit intimal hyperplasia in vitro[46] and in vivo[43]. Therefore, detailed studies on the timing of PCNA expression after coronary angioplasty in humans will be important for the development of therapeutic maneuvers in the future.

Previous reports[11,15] indicated that clinical predictors of restenosis after DCA included cholesterol levels (≥ 200 mg/dl), hypertension, a long lesion (≥ 10 mm), small vessel diameter (<3mm),

a noncalcified lesion, and the use of a smaller (6F) catheter. However, the histological predictors of restenosis after DCA have not been thoroughly delineated. It was reported that increased expression of the B isoform of nonmuscle myosin heavy chain was a high risk for restenosis after atherectomy[44]. In contrast, previous studies[22,41,52] on DCA sample tissue have not revealed any histologic features to be correlated to subsequent restenosis. In particular, Miller et al[22] reported that subsequent restenosis after DCA did not correlate with the presence of intimal hyperplasia in the primary coronary lesions. Taylor et al[48] reported that the presence of proliferating cells (PCNA labeled) in coronary lesions did not predispose patients to restenosis after atherectomy. This was consistent with our findings. This evidence suggests that PCNA expression is not reflective of the proliferative potential or subsequent lesion progression. However, while accurate predictions for individual patients and lesions are not yet possible, a larger study may help characterize this relationship more definitively.

In summary, despite the DCA sampling limitations of studying tissues obtained by DCA[1,7,8,13,56], we found a high incidence of intimal hyperplasia, plaque morphology of SMC proliferation, and high levels of PCNA expression in the primary tissue samples obtained from patients with ACS and postangioplasty tissue samples compared with those in the primary tissue samples from patients with SAP. Moreover, neovascularization, hemorrhage, and inflammation in plaques were evident in the plaque of primary ACS samples. Accordingly, the precipitating stimulus leading to cellular proliferation in primary ACS lesions may be caused by these plaque morphological changes, although that in postangioplasty lesions is caused by mechanical injury. Our findings suggest that excessive proliferation of vascular cells, particularly SMCs is involved in the pathogenesis of ACS and in the process of wound repair after angioplasty in humans.

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