Increased adrenomedullin immunoreactivity and mRNA expression in coronary plaques obtained from patients with unstable angina

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Objective: To examine the expression and localisation of adrenomedullin in human coronary atherosclerotic lesions from patients with unstable angina (UAP) and stable angina (SAP), and to study the relation between adrenomedullin expression and plaque instability.

Design: A retrospective observational study.

Patients: Directional coronary atherectomy samples were obtained from 15 patients with UAP and 12 with SAP.

Methods: The localisation of adrenomedullin was examined by immunohistochemistry, and adrenomedullin mRNA expression was measured by quantitative polymerase chain reaction.

Results: Adrenomedullin immunoreactivity was preferentially localised in macrophages, intimal smooth muscle cells, and proliferated microvessels. The mean number of adrenomedullin positive cells in five high power fields (×400) per specimen was higher in patients with UAP than in those with SAP (mean (SEM), 110 (13) v 76 (7); p < 0.05), and the ratio of adrenomedullin positive to total cells was higher in patients with UAP (43.0 (2.2)% v 34.2 (2.0)%; p < 0.01). More adrenomedullin mRNA was expressed in the plaque of patients with UAP than in those with SAP (60.4 (16.9)% v 9.7 (3.3)%; p < 0.01).

Conclusions: The findings suggest that adrenomedullin is involved in the development of atherosclerosis and plaque instability in human coronary arteries, in an autocrine or paracrine manner.
4°C. In addition, intimal smooth muscle cells, macrophages, and intraplaque microvessels were identified using anti-α-actin antibody (HHF35), anti-CD68 antibody, and anti-CD34 antibody, respectively (Dako Japan, Kyoto, Japan). Intervening washes with phosphate buffered saline (PBS) were followed by an incubation with EnVision+ (Dako Japan) for 30 minutes at room temperature. After further washes in PBS, the sections were incubated with 0.05% 3,3′-diaminobenzidine containing hydrogen peroxide, and counterstained with Meyer's haematoxylin.

Three independent observers who were blind to the patients identities manually counted the number of nuclei of adrenomedullin positive and negative cells in five high power fields (× 400) per specimen, as previously described. The results of these quantitative analyses are reported as the ratio (%) of positively stained cells among the five high power fields.

**Adrenomedullin mRNA measurement by real time quantitative polymerase chain reaction**

Individual atherectomy specimens from 14 of the 27 patients with UAP (n = 6) or SAP (n = 8) were frozen in liquid nitrogen and stored at −80°C. Total RNA (1 μg) was extracted from frozen specimens using isolation reagent16 (Trizol, Life Technologies, Rockville, Maryland, USA), was reverse transcribed employing SuperScript reverse transcriptase (Gibco-BRL, Life Technologies, Gaithersburg, Maryland, USA) into cDNA. Human adrenomedullin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA concentrations were quantified by a novel quantitative polymerase chain reaction (PCR) method, real time quantitative PCR (Prism 7700 Sequence Detector, Applied Biosystems, Foster City, California, USA), as previously reported. The mean numbers of adrenomedullin positive and negative cells in five high power fields (× 400) per specimen were significantly higher in patients with SAP than in those with SAP (43.0 (2.2)% v 34.2 (2.0)%; p < 0.01) (fig 3). The expression of adrenomedullin mRNA was significantly higher in patients with SAP than in those with SAP (60.4 (16.9)% v 9.7 (3.3)%; p < 0.01) (fig 4).

**DISCUSSION**

This study shows first, that adrenomedullin immunoreactivity of the coronary plaque is present in macrophages, intimal smooth muscle cells, and proliferating microvessels; second, that the amount of adrenomedullin immunoreactivity is significantly higher in patients with UAP than with SAP; and third, that expression of adrenomedullin mRNA is greater in plaque from patients with UAP than with SAP. These results suggest that adrenomedullin is involved in the pathogenesis of plaque instability of human coronary atherosclerosis.

Previous studies have determined that adrenomedullin has multifunctional biological activities, including vasorelaxation, diuretic action, and inhibition of aldosterone secretion. Circulating adrenomedullin concentrations are raised in cardiovascular, endocrine, renal, and thrombotic diseases. Nakayama and colleagues reported that macrophages in human aortic atherosclerotic lesions, as well as endothelial cells and vascular smooth muscle cells, were immunopositive for adrenomedullin. However, few studies have addressed the possible role of adrenomedullin in the pathogenesis or process of atherosclerosis. Our present results are consistent with Nakayama’s, but we are the first to observe adrenomedullin immunoreactivity in coronary atherosclerotic lesions.

Macrophages play a crucial role in atherogenesis and are highly activated in advanced atherosclerotic lesions. Macrophage-rich areas are more common in atheromatous plaque, especially in its shoulder regions, among patients with UAP. Activated macrophages express inflammatory cytokines and matrix metalloproteinases which might induce plaque instability. Thus macrophage-rich lesions are considered a rupture prone region of atheromatous plaque, capable of causing acute coronary syndromes. Cultured or

**Table 1 Baseline clinical characteristics of the study population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SAP (n = 12)</th>
<th>UAP (n = 15)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63 (3)</td>
<td>63 (2)</td>
<td>0.97</td>
</tr>
<tr>
<td>Male/female</td>
<td>11/1</td>
<td>14/1</td>
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<td>Systemic hypertension</td>
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<td>8</td>
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<td>Hyperuricaemia</td>
<td>2</td>
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<td>&gt;0.99</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4</td>
<td>6</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Smoker</td>
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<tr>
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<tr>
<td>Family history</td>
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<tr>
<td>LAD</td>
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<td>13</td>
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<tr>
<td>RCA</td>
<td>2</td>
<td>2</td>
<td>0.39</td>
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<tr>
<td>Aspirin</td>
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<td>15</td>
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</tr>
<tr>
<td>Stains</td>
<td>3</td>
<td>5</td>
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</tbody>
</table>

Values are mean (SEM) or n.

*pPrevious diagnosis of hyperlipidaemia or patient on lipid lowering therapy
1Body mass index >30 kg/m2
2A parent, sibling, or child with a history of coronary artery disease
3LAD, left anterior descending coronary artery; LMT, left main trunk; RCA, right coronary artery; SAP, stable angina pectoris; UAP, unstable angina pectoris.

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Figure 1  Representative histological features of atherectomy samples from patients with unstable angina pectoris (UAP) and stable angina pectoris (SAP). Foam cells and spindle cells accumulate in an atherectomy sample from a patient with UAP (A). Many of these cells (60% in this field) are positively stained for adrenomedullin (B). Plaque tissue from a patient with SAP is fibrous (C), and immunoreactivity for adrenomedullin is weak in some spindle cells (30% in this field) (D). AM, adrenomedullin; HE, haematoxylin and eosin stain.

Figure 2  Representative immunohistochemical features of atheromatous plaque in a patient with unstable angina pectoris. Many adrenomedullin positive stellate or spindle shaped cells are intimal smooth muscle cells (A, B). Adrenomedullin positive foam cells are macrophages (C, D). In neovascularised microvessels, endothelial cells are immunopositive for adrenomedullin (E, F). AM, adrenomedullin.
Increased adrenomedullin immunoreactivity and mRNA expression

peritoneal macrophages produce and secrete adrenomedullin, and both types of cell are stimulated by lipopolysaccharide, interferon-γ, and tumour necrosis factor-α. Moreover, adrenomedullin expression and secretion by the monocyte–macrophage lineage can be regulated by the degree of differentiation into macrophages, and adrenomedullin inhibits the release of chemokines from macrophages. These findings suggest that adrenomedullin secreted from macrophages in atherosclerotic lesions could inhibit the progression of atherosclerosis and prevent plaque disruption. However, we could not define the pathophysiological role of adrenomedullin expressed in macrophages in the present study.

Vascular smooth muscle cells in atherosclerotic lesions were also positively stained for adrenomedullin in this study. Intimal smooth muscle cells have unique properties that are not found in medial cells, such as high mitotic activity and growth factor receptor expression, and the phenotypes of intimal smooth muscle cells could be different from those of medial smooth muscle cells. Although the roles of adrenomedullin expressed in intimal smooth muscle cells have not been intensively studied, recent reports suggest that adrenomedullin suppresses the proliferation and migration of cultured vascular smooth muscle cells, and endothelin production in cultured vascular smooth muscle cells.

Furthermore, local adrenomedullin gene delivery significantly inhibits arterial thickening, promotes re-endothelialisation, and increases vascular cGMP concentrations in the rat artery after balloon angioplasty. Vascular injury is obvious and concomitant with increased oxidative stress induced by angiotensin II/salt loading in knockout adrenomedullin +/- mice in which only the adrenomedullin peptides are disrupted. These lines of evidence suggest that adrenomedullin plays a protective role against the development of atherosclerosis.

Plasma adrenomedullin concentrations of the coronary sinus and peripheral vein increase immediately after percutaneous transluminal coronary angioplasty. The increased concentrations of plasma adrenomedullin are thought to originate from injured atheromatous plaque or from the ischaemic myocardium. Levels of adrenomedullin immunoreactivity and mRNA are also high in human failed left ventricular muscle, and plasma adrenomedullin is increased in chronic heart failure and acute myocardial infarction. Our results also suggest that some of the increased plasma adrenomedullin could be released from the injured atheromatous plaque by percutaneous coronary intervention, especially in patients with UAP. Further investigation is needed to clarify this hypothesis.

The limitations of our study are the small number of subjects, the lack of randomisation to a control group, and a bias towards coronary lesions suitable for an atherectomy procedure rather than balloon angioplasty or stent deployment. Nonetheless, our paper is the first to show that levels of adrenomedullin immunoreactivity and mRNA expression are higher in atherectomy samples from patients with UAP than with SAP. Although we could not determine the roles of adrenomedullin in atherosclerotic lesions in this study, our results suggest that adrenomedullin may be involved in plaque instability in an autocrine or paracrine manner.

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