Human gastroepiploic artery has greater chymase activity than the internal thoracic artery

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Abstract

Objective: Recent reports have demonstrated that long-term patency of the gastroepiploic artery (GEA) in coronary artery bypass grafting (CABG) is less satisfactory compared with the internal thoracic artery (ITA). However, the reason has not been fully elucidated. Angiotensin II is known to play an important role in the development of intimal hyperplasia, we hypothesized that the GEA is different from the ITA with respect to angiotensin II-forming ability. Accordingly, we measured activities of angiotensin II-forming enzymes, angiotensin-converting enzyme (ACE) and chymase, in human GEA and ITA.

Methods: Remnant of the GEAs and ITAs were obtained from 24 patients who underwent CABG in which both conduits were used simultaneously. Activities of ACE and chymase were measured by using the extract form the GEA or ITA. Sections of the GEA or ITA were immunohistochemically stained with anti-human chymase antibody.

Results: The ACE activity of the GEA (0.28 ± 0.16 mU/mg protein) was greater than that of the ITA (0.18 ± 0.11, p < 0.001). The chymase activity of the GEA (11.11 ± 7.15 mU/mg protein) was also greater than that in the ITA (7.13 ± 4.89, p < 0.001). The density of chymase-positive cells in the GEA (3.8 ± 4.2 cells/mm²) was greater than that in the ITA (1.1 ± 1.2, p < 0.01).

Conclusion: Activities of both ACE and chymase were significantly greater in the GEA compared with the ITA. The GEA may be different from the ITA with respect to potential ability of angiotensin II-formation.

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1. Introduction

The left internal thoracic artery (LITA) has been a mainstay as a conduit in coronary artery bypass grafting (CABG) because of histological advantage and its excellent long-term patency [1,2]. Recently, the right gastroepiploic artery (GEA) has been used as a second choice of an arterial conduit, especially when multiple revascularizations are scheduled [3–5]. Since the GEA can be used as an in situ conduit same as the ITA, it is preferable in CABG without aortic manipulation to prevent cerebral infarction and aortic dissection. However, recent reports have shown that long-term patency of the GEA is less satisfactory compared with the LITA [6–8]. The reason for this has not been clearly elucidated.

Angiotensin II plays an important role in occlusion of native coronary artery and graft conduits after intervention [9]. Angiotensin II is produced from Ang I mainly by chymase and angiotensin converting enzyme (ACE) and is known to stimulate growth of the smooth muscle cells and extracellular matrix, which might result in intimal hyperplasia. Recently, we have found that chymase activity in the saphenous vein was significantly greater than that in the ITA [10]. However, little is known about the differences in angiotensin II-forming ability between the GEA and ITA. Accordingly, we hypothesized that the GEA has greater angiotensin II-forming ability than the ITA, resulting in more frequent graft occlusion or stenosis. In the present study, we assessed the activity of angiotensin II-forming enzymes, ACE and chymase, and the distribution of chymase-positive mast cells in human ITA and GEA.

2. Materials and methods

2.1. Sampling of the GEAs and the ITAs

Remnant of the GEAs and ITAs were obtained from 24 patients who underwent CABG in which both types of arterial conduits were used simultaneously. All grafts were
skeletonized using an ultrasonic scalpel. There were 18 male and 6 female, ranging in age from 49 to 77 years. ACE inhibitor or angiotensin II receptor blocker (ARB) was administrated in 8 or 6 patients, respectively. Written consent was obtained from all patients after a full explanation of this study. The protocol of this study complied with the principles of the Helsinki Declaration.

2.2. Measurement of enzyme activities in vascular tissue

The ITA or GEA specimens were minced and homogenized in 20 mmol/l Na-phosphate buffer (pH 7.4). The homogenate was centrifuged at 20,000 \(\times\) g for 30 min. The pellet was homogenized in 10 mmol/l Na-phosphate buffer (pH 7.4), containing 2 mol/l KCl and 0.1% Nonidet P-40. The homogenate was centrifuged at 20,000 \(\times\) g for 30 min. The supernatant was used for measurement of the ACE and chymase activities [10].

The ACE activity was measured with use of a synthetic substrate, hippuryl-His-Leu, specifically designed for ACE as previously reported [10]. The chymase activity was measured by incubating tissue extracts with angiotensin I, as described previously [10].

2.3. Histologic analysis

The ITA or GEA segments were fixed in Carnoy’s solution, embedded in paraffin, and sectioned 3 \(\mu\)m in thickness. The sections were stained with hematoxylin–eosin and van Gieson elastin stain, respectively. For immunohistochemical staining, mouse anti-human chymase monoclonal immunoglobulin G (1:1000 dilution; Chemicon International Inc.) was used as a primary antibody to detect chymase. Immunohistochemical staining was performed using an avidin–biotin–peroxidase kit (Dako LSAB kit; DAKO Corporation) with 3-amino-9-ethylcarbazole color development. The number of chymase-positive cells was counted and the area of the arterial wall was quantified with an image-analysis software (Scion image Ver. 1.63).

3. Statistical analysis

All data were expressed as mean \(\pm\) SD. Comparisons between two conduits were made by the 2-tailed Student’s \(t\)-test for paired observations. Clinical factors influencing the each level of ACE and chymase activities were investigated with univariate analysis.

4. Results

There was no mortality and morbidity. Postoperative angiography was performed prior to discharge in 22 of 24 patients and revealed 100% patency in all grafts.

4.1. Enzymatic activities of ACE and chymase of the ITA and GEA

In all 24 patients, the ACE activity of the GEA (0.28 \(\pm\) 0.16 mU/mg protein) was significantly greater than that of ITA (0.18 \(\pm\) 0.11 mU/mg protein, \(p = 0.0009\)). The chymase activity in the GEA (11.11 \(\pm\) 7.15 mU/mg protein) was significantly greater than that in the ITA (7.13 \(\pm\) 4.89 mU/mg protein, \(p = 0.0007\)), as shown in Fig. 1. Excluding 14 patients with ACE inhibitor or ARB administration preoperatively, both the ACE (0.24 \(\pm\) 0.12 mU/mg protein) and chymase (12.21 \(\pm\) 9.91 mU/mg protein) activities in GEA were also significantly greater than those in ITA (ACE; 0.12 \(\pm\) 0.06 mU/mg protein, \(p = 0.006\), chymase; 7.21 \(\pm\) 6.08 mU/mg protein, \(p = 0.018\), respectively).

Univariate analysis revealed that the chymase activities in both the ITA and GEA in patients with chronic renal dysfunction (\(n = 2,\) serum creatinine >1.2 mg/dl) were significantly greater than those in patients with normal creatinine level (\(n = 22,\) ITA; \(p = 0.012,\) GEA; \(p = 0.008\)), whereas the ACE activities in these grafts were not significantly different. In two patients with renal dysfunction, one was on chronic hemodialysis and the other had hypertension and peripheral arterial disease. Other variables, such as age, gender, unstable angina, left main trunk disease, 3-vessel disease, hypertension, hyperlipidemia, diabetes mellitus, peripheral vascular disease, left ventricular ejection fraction less than 50%, and history of percutaneous coronary intervention did not affect the level of the ACE and chymase activity.

4.2. Histochemical study in ITA and GEA

Immunostaining with an antibody to human chymase localizes in the adventitial layers in both the GEA and ITA (Fig. 2). The density of the chymase-positive cells in the GEA (3.8 \(\pm\) 4.2 cells/mm²) was significantly greater than that in the ITA (1.1 \(\pm\) 1.2, \(p = 0.004\)).
5. Discussion

Although early patency of the GEA (more than 90%) compares favorably with the ITA, long-term patency of the GEA is less satisfactory [6–8]. The possible mechanism of the relatively poorer long-term patency of the GEA was suspected by Suma et al. [7] as followed: (1) the GEA graft might have flow competition with native coronary artery which had less critical stenosis and (2) the flow through the GEA graft might be small when the graft was anastomosed to the coronary artery with poor run off distally to the stenosis. Contrary, Hirose et al. [8] have advocated that despite the avoidance of using the GEA to revascularize mildly stenosed coronary artery, patency at 5 years after CABG is inferior to the ITA. In addition, early patency of the GEA is decreased to 80% when used as a free graft [6]. Although it has been reported that differences in histologic and physiologic characteristics between the GEA and the ITA might affect long-term patency, precise mechanisms of late occlusion of the GEA still remain unclear [6–8].

In the present study, we have demonstrated for the first time that both the ACE and chymase activities in the GEA were significantly greater compared with the ITA.

Intimal hyperplasia is basal component in the development of atherosclerosis found in the graft stenosis and occlusion. Angiotensin II is known to play an important role to induce the intimal hyperplasia consisting of accumulation of smooth muscle cells and extracellular matrix [9]. In rat models of arterial injury, angiotensin II induces smooth muscle cell proliferation and ACE inhibitor prevents neoointimal formation [11]. On the other hand, ACE inhibitor failed to prevent intimal hyperplasia of the bypass graft in baboon and restenosis of the coronary artery after percutaneous coronary intervention (PCI) in human [12]. These controversial findings suggest that there may be different mechanisms or systems among species to induce angiotensin II-formation causing intimal hyperplasia. While ACE is the only enzyme inducing angiotensin II-formation in rat vascular tissue, chymase system in addition to ACE is another major pathway producing angiotensin II in the tissues of human, monkey, dog, and hamster [13]. Supporting these findings, it is reported that ARB prevented restenosis after PCI in human [14]. Moreover, it also reported effect of chymase inhibitors on reducing intimal hyperplasia in artery injured by balloon and in bypass graft in dog [13,15]. Accordingly, chymase-dependent angiotensin II-formation may play an important role in intimal hyperplasia and atherosclerosis in human, and the high level of angiotensin II-formation ability in the GEA may be one reason for the poorer late patency, when it is used as a graft conduit.

Mast cells are known to accumulate in the atheromatous lesions of the human arteries, suggesting that they play a pathological role in the development of atherosclerosis [16]. Chymase-containing mast cells have been found to be located in the adventitia of aorta, and the density of chymase-containing mast cells in the atherosclerotic aorta was higher than that in the normal aorta [17]. We have found that chymase-positive mast cells were located in both the media and adventitia in the saphenous vein, while those were located only in the adventitia in ITA [10]. In the present study, chymase-positive mast cells were located in the adventitia in both the ITA and the GEA, and the density of mast cells in the GEA was significantly greater than that in the ITA. Thus, the difference in the level of the chymase may be due to the density of mast cells containing chymase.

Another novel finding in this study is that the chymase activity, but not the ACE activity, in patients having more than 1.2 mg/dl of serum creatinine level was significantly higher than that in patients with normal creatinine level, in both ITA and GEA. Patients on chronic hemodialysis showed high incidence of arteriosclerotic cardiovascular mortality or morbidity and high rate of restenosis after PCI [18]. These reports suggest that the process of atherosclerosis may be accelerated in hemodialysis patients. Recently, chymase in kidney tissue has been found to be markedly upregulated in the diabetic and hypertensive nephropathy [19]. Renal function may be linked to the arterial chymase activity, and that the arterial chymase activity may be upregulated in patients with renal dysfunction.

In conclusion, the present study demonstrated for the first time that both the ACE and chymase activities in the GEA were significantly greater than those in the ITA. The high levels of Ang II-forming ability in the GEA may be associated with more frequent graft stenosis or occlusion than the ITA.
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References