Differences Between Patients with and without Atherosclerosis in Expression Levels of Inflammatory Mediators in the Adipose Tissue Around the Coronary Artery

Tomoaki Suzuki, MD, Hisakazu Ogita, MD, Akira Sato, PhD, Naoshi Minamidate, MD and Kohei Hachiro, MD

Summary

Perivascular adipose tissue (PVAT) secretes large amounts of inflammatory mediators and plays a certain role in atherosclerosis formation from the exterior of the vessel. In the present study, we examined the expression level of inflammation-related mediators using adipose tissue samples harvested from patients with and without coronary artery disease (CAD). The subjects were 23 patients who underwent elective coronary bypass surgery (CAD group) and 17 patients who underwent elective mitral valve surgery (non-CAD group) between January 2017 and March 2018. The adipose tissue was harvested from three sites: the ascending aorta (AO), subcutaneous fat (SC), and pericoronary artery (CO) for the measurement of the expression levels of interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor (TNF)-α, interferon (INF)-γ, and arginase (Arg)-1. In both the non-CAD and CAD groups, the expression levels of all mediators, except Arg-1, which showed a tendency to have higher levels in the SC than in the AO and CO, tended to upregulate in the AO than in the SC and CO. The CAD group had higher values of almost all mediators, except Arg-1. Most importantly, the expression levels of IL-1β, IL-6, and IL-10 in the coronary artery were significantly higher in the CAD group. The expression levels of inflammatory mediators in the pericoronary adipose tissue were significantly higher in the CAD than in the non-CAD group. The adipose tissue appears to influence atherosclerosis formation from the exterior of the coronary artery.

Key words: Perivascular fat, Coronary artery disease, Mediator, Interleukin, Biomarker

Nowadays, it is widely known that perivascular adipose tissue (PVAT) influences the progression of atherosclerosis from the exterior of the vessel.1,2 In the last two decades, researchers have studied this problem in terms of both the quality and quantity of the adipose tissue using computed tomography imaging and have established a significant correlation between the volume of pericardial adipose tissue and the severity of coronary atherosclerosis.3-5 PVAT has been recognized as an endocrine organ that releases a number of functional mediators such as cytokines and adipokines.6 Under healthy conditions, this release of cytokines and adipokines may exert an anti-atherosclerotic effect and help maintain normal vasodilatory function.7 Dysfunctional adipose tissue, however, such as in obese individuals, releases inflammatory and pro-inflammatory cytokines from the exterior of the vascular vessel and is associated with coronary disease.8,9 Especially in patients with coronary artery disease (CAD), inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, are expressed at high levels in the surrounding adipose tissue.10-12 IL-10 is well-known as the most potent anti-inflammatory cytokine that can be released by T cells, macrophages, and monocytes.13,14 Arginase (Arg)-1 is secreted by group 2 innate lymphoid cells, which act as key initiators of immunity and type 2 inflammation.15 Arg-1 activity is also known as a kind of signature of alternatively activated macrophages.16 Interferon (INF)-γ is known to be released by natural killer cells that are identified in PVAT. Natural killer cells regulate visceral adipose tissue inflammation via IFN-γ release.17

In the present report, we evaluated the difference in cytokine expression between patients with and without CAD using adipose tissue samples harvested during coronary artery bypass or mitral valve surgery.

Methods

Study population: The present study was prospectively conducted and approved by the institutional review board of Shiga University of Medical Science (R2016-122). The subjects were 23 patients who underwent elective coro-
nary bypass surgery (CAD group) and 17 patients who underwent elective mitral valve surgery (non-CAD group) between January 2017 and March 2018. Informed consent was obtained from each patient.

**Adipose tissue harvest:** Adipose tissue samples were obtained from three sites at the beginning of the surgery before cardiopulmonary bypass was started. Subcutaneous adipose samples were taken from the subcutaneous fat (SC) between the skin and the lower end of the sternum. Periaortic adipose samples were taken from the medial ascending aorta (AO). Pericoronal artery (CO) adipose samples were taken from around the proximal right coronary artery at the atherosclerotic site. Each adipose sample was immediately frozen in liquid nitrogen and stored at −80°C for mRNA isolation and real-time polymerase chain reaction.

**Total RNA extraction from adipose tissue samples:** Total RNA was extracted from each adipose sample using NucleoSpin RNA (MACHEREY-NAGEL, Düren, Germany).

**qPCR:** After total RNA extraction, cDNA was synthesized using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan). qPCR was performed using the LightCycler Instrument (Roche Diagnostics, Basel, Switzerland). The samples were run in duplicate, and data were quantified using the standard curve method. The primers used were as follows: IL-6 (forward 5′-TACCTTCCAGGAGATTTC-3′ and reverse 5′-TTTTCTGGCCAGTGCCTTT-3′), IL-1β (forward 5′-GGGCCCTAAGGAAAGAATC-3′ and reverse 5′-TTTCTGTTGAAGGTTGCTGA-3′), TNF-α (forward 5′-CAGAGGGCTGTACCTCATC-3′ and reverse 5′-GGAAGACCCCTCCCAGATAG-3′), IFN-γ (forward 5′-TGACCAGAGCATCCAAAAGA-3′ and reverse 5′-CTTCTTGACCTCGAAACAGC-3′), IL-10 (forward 5′-TGCTTCTCAGAGTGAAGA-3′ and reverse 5′-GGTCTTGGTTCTCAGCTTG-3′), Arg-1 (forward 5′-GGCTTGCTGCTGAGAAAC-3′ and reverse 5′-ATTGCCCCAATCGTTGCTCC-3′), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (forward 5′-CCTGTTCGACAGTCAGCCG-3′ and reverse 5′-GGAAGACCCCTCCCAGATAG-3′) and glyceraldehyde.

**Statistical analysis:** Continuous variables are expressed as mean ± standard error of mean. The variables were compared using the Student’s t test and one-way ANOVA [SPSS 22.0 for Windows, SPSS Chicago, Illinois]. Differences were considered significant at P value of < 0.05.

**Results**

**Patient characteristics and laboratory measurements:** Patient characteristics and laboratory measurements are listed in Table I. There was no significant difference in age or body mass index between the CAD and non-CAD groups. The CAD group had a high prevalence of smoking history and diabetes mellitus. Of the laboratory values, serum creatinine and triglycerides were significantly higher in the CAD than in the non-CAD group. As for medications, statins and antiplatelet agents were more used in the CAD than in the non-CAD group.

**Expression of inflammatory mediators in the SC, AO, and CO:** The Figure and Table II show the expression of each inflammatory biomarkers (IL-1β, IL-6, IL-10, TNF-α, INF-γ, and Arg-1) in the SC, AO, and CO. GAPDH was used as the internal control. Data are presented as the ratio of mediator expression to GAPDH expression. The data allowed a number of new findings. Even in the same individual, the biomarker expression levels differed according to the location from which the adipose tissue was harvested. In both the non-CAD and CAD groups, the expression levels of all mediators upregulated in the AO than in the SC and CO, except Arg-1, which tended to have higher levels in the SC than in the AO and CO. In the non-CAD group, the expression levels of IL-1β (P = 0.04), IL-10 (P = 0.04), TNF-α (P ≤ 0.01), and INF-γ (P = 0.02) were significantly higher in the AO than in the SC (Table II). In the CAD group, the expression level of TNFα (P < 0.01) was significantly higher in the AO than in the SC, while the expression level of Arg-1 (P = 0.05) was significantly lower in the CO than in the SC (Table II). The Figure also shows that almost all values, except Arg-1, tended to be higher in the CAD than in the non-CAD group. Most importantly, the expression levels of IL-1β (P = 0.02), IL-6 (P = 0.03), and IL-10 (P = 0.009) were significantly higher in the CAD group.

**Discussion**

Human adipose tissue is broadly classified into two types: subcutaneous and visceral. It is well-known that the visceral adipose tissue is associated with lifestyle disease such as metabolic syndrome, dyslipidemia, diabetes mellitus, and hypertension, causing atherosclerotic cardiovascular disease. In the last two decades, the adipose tissue has been studied at other sites, including the perivascular area, skeletal muscle, and liver. To distinguish them from subcutaneous or visceral adipose tissue, these adipose tissues are called “ectopic adipose tissue” or “third adipose tissue.” Ectopic adipose tissues have also been found to be associated with the risk of atherosclerotic cardiovascular events. PVAT has been especially well investigated in terms of both quantity and quality and revealed to have a strong influence on the progression of atherosclerosis. Nowadays, PVAT is regarded as an endocrine organ that secretes various inflammatory or pro-inflammatory mediators that influence atherosclerosis formation from the artery exterior. Normal PVAT plays an important role in maintaining vascular homeostasis and anti-atherosclerotic effect by producing protective substances such as adiponectin. Dysfunctional adipose tissue, such as in obese individuals, releases inflammatory and pro-inflammatory cytokines from the coronary artery exterior and is associated with CAD.
higher than in subcutaneous adipose tissue in patients undergoing coronary bypass surgery. Lacobellis and coworkers compared the expression levels of adipocytokines in epicardial adipose tissue samples between patients with and without CAD. They found that anti-inflammatory adiponectin expression was significantly lower in patients with CAD than in those without CAD, suggesting that an imbalance of inflammation may occur in the adipose tissue around atherosclerotic coronary artery. Hirata and coworkers also compared the expression levels of adipocytokines in epicardial adipose tissue between 38 patients with and without CAD using the pericoronary adipose tissue. The distinctive expression pattern of the other mediators, Arg-1 expression was not higher in the CAD group than in the non-CAD group. The adipose tissue in the SC tended to show higher Arg-1 expression levels than in the AO and CO, which was quite different from the expression pattern of the other mediators. Arg is known as a key element of the urea cycle that converts L-Arg to urea and L-ornithine, mainly in the liver. L-Arg is an important substrate for nitric oxide synthase, and the enzyme Arg-1 is also closely related to nitric oxide synthase, which directly reacts with vessel homeostasis. Recently, it was found that Arg-1 is secreted by group 2 innate lymphoid cells, which act as key initiators of immunity and type 2 inflammation. In the present study, the not particularly high level of Arg-1 expression in the CO in the CAD group may indicate that the alternatively activated macrophage is not closely related to the inflammation balance in the pericoronary adipose tissue. The distinctive expression pattern of Arg-1 is not well understood and requires further investigation.

Thirdly, the expression level of IL-10 in the CO was significantly higher in the CAD than in the non-CAD group. This finding is very valuable as almost no in vivo measurement of Arg-1 expression levels, have not been previously reported.

In the present study, we similarly compared cytokine expression between patients with and without CAD using adipose tissue samples harvested during coronary bypass or mitral valve surgery. Some aspects of our approach, including the harvesting of periaortic adipose tissue and the measurement of Arg-1 expression levels, have not been previously reported.

We made a number of interesting findings. Firstly, in all areas (SC, CO, and AO), the expression levels of all mediators, except Arg-1, had an upregulated tendency in the CAD group than in the non-CAD group. It might be expected that individuals with atherosclerotic disease would have a higher level of inflammatory reaction in adipose tissues throughout the body than those without.

Secondly, the pattern of Arg-1 expression was quite different from that of other mediators. Unlike the other mediators, Arg-1 expression was not higher in the CAD group than in the non-CAD group. The adipose tissue in the SC tended to show higher Arg-1 expression levels than in the CO and AO, which was quite different from the expression pattern of the other mediators. Arg is known as a key element of the urea cycle that converts L-Arg to urea and L-ornithine, mainly in the liver. L-Arg is an important substrate for nitric oxide synthase, and the enzyme Arg-1 is also closely related to nitric oxide synthase, which directly reacts with vessel homeostasis. Recently, it was found that Arg-1 is secreted by group 2 innate lymphoid cells, which act as key initiators of immunity and type 2 inflammation. In the present study, the not particularly high level of Arg-1 expression in the CO in the CAD group may indicate that the alternatively activated macrophage is not closely related to the inflammation balance in the pericoronary adipose tissue. The distinctive expression pattern of Arg-1 is not well understood and requires further investigation.

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**Figure.** A: Differences of the expression levels of IL-1β and IL-6 between patients with and without CAD in the SC, AO, and CO. B: Differences of the expression levels of IL-10 and TNF-α between patients with and without CAD in the SC, AO, and CO. C: Differences of the expression levels of INF-γ and Arg-1 between patients with and without CAD in the SC, AO, and CO. SC indicates subcutaneous; AO, aorta; and CO, coronary.
data on IL-10 expression in the pericoronary adipose tissue have been previously reported. IL-10 is well-known as the most potent anti-inflammatory cytokine and can be produced in response to inflammation signals by various immune cells, including T cells, macrophages, and monocytes. In the present study, IL-10 was expressed at high levels in patients with CAD, along with other pro-inflammatory cytokines, including IL-6 and IL-1β. This may indicate that IL-10 plays an important role in counteracting inflammation in the pericoronary adipose tissue in response to high expression levels of inflammatory mediators causing atheromatous progression.

Fourthly, and most importantly, the expression levels of IL-6 and IL-1β in the CO were higher in the CAD than in the non-CAD group. This result confirms the findings of past investigators. Some kind of inflammation must occur in the adipose tissue surrounding the atherosclerotic coronary artery. However, whether adipose tissue inflammation occurs after the appearance of atherosclerotic coronary artery or whether prior inflammation is atheromatous progression. In either case, our findings are valuable as there are very few existing in vivo data sets that compare patients with and without atherosclerosis.

Limitations: The sample size was small, and we did not analyze the relationship between clinical background (age, sex, body size, hypertension, dyslipidemia, etc.) and experimental data, nor account for the influence of statin therapy on the development of inflammatory reaction in the adipose tissue, which may be affected by these two factors.

Conclusion

In the present study, we compared cytokine expression between patients with and without CAD using adipose tissue samples harvested during coronary bypass or mitral valve surgery. The expression levels of IL-6 and IL-1β in the CO were higher in the CAD than in the non-CAD group. Some kind of inflammation must occur in the adipose tissue surrounding the atherosclerotic coronary artery. Adipose tissue appears to influence atherosclerosis formation from the exterior of the coronary artery.

Disclosure

Conflicts of interest: All author has no conflicts of interest.

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