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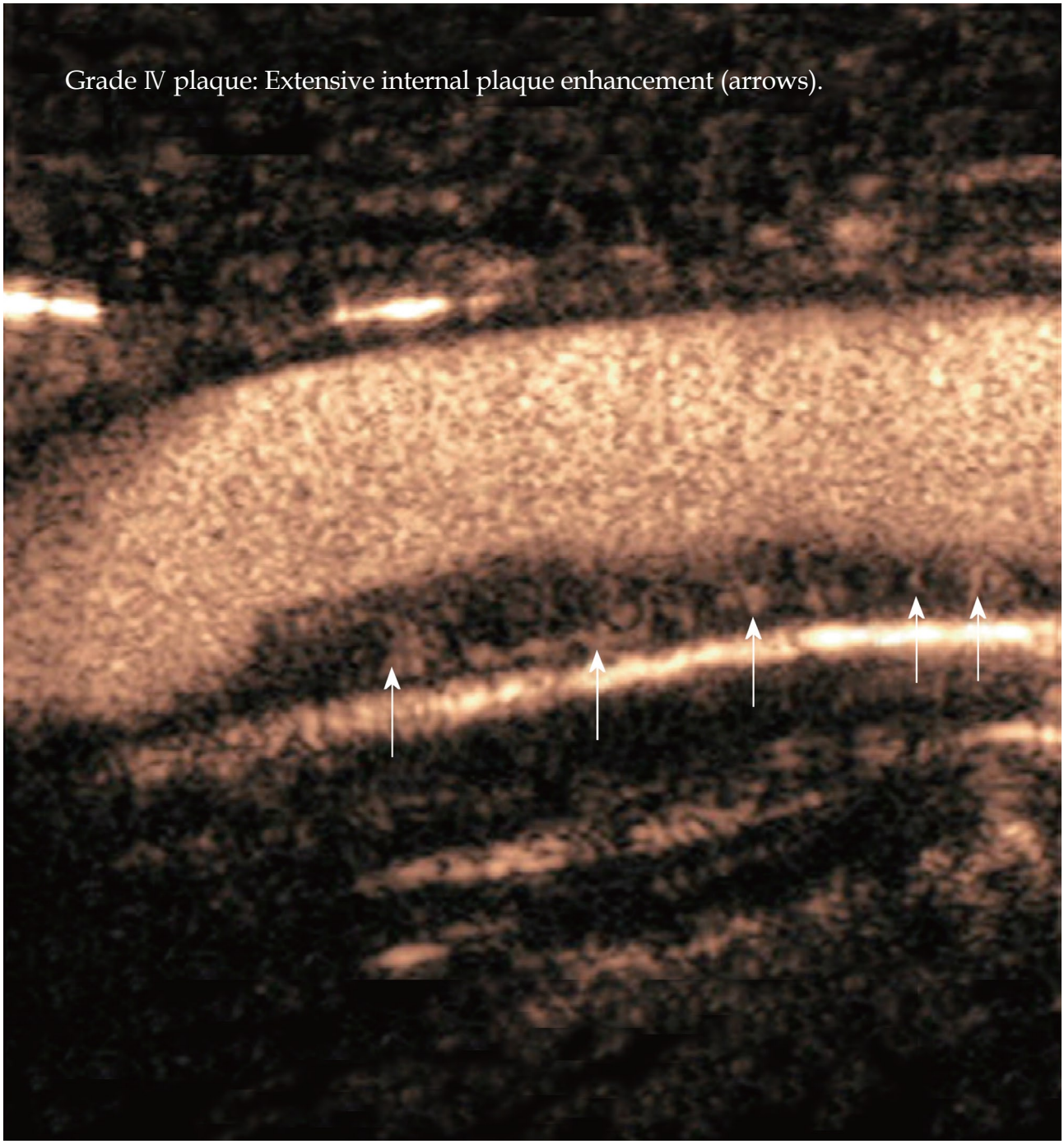

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EDITORIAL

Cardiovascular and metabolic effects of Berberine

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Abstract

Berberine (BBR) is a natural alkaloid isolated from the Coptis Chinensis. While this plant has been used in Ayurvedic and Chinese medicine for more than 2500 years, interest in its effects in metabolic and cardiovascular disease has been growing in the Western world in the last decade. Many papers have been published in these years reporting beneficial effects in carbohydrate and lipid metabolism, endothelial function and the cardiovascular system. In this review, we report a detailed analysis of the scientific literature regarding this topic, describing the effects and the underlying mechanisms of BBR on carbohydrate and lipid metabolism, endothelial function and the cardiovascular system.

Key words: Metabolic syndrome; Berberine; Adenosine monophosphate kinase; Insulin resistance; Hyperlipidemia; Endothelial function

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INTRODUCTION

Berberine (BBR) is a natural alkaloid isolated from the Coptis Chinensis. This plant has been used for medicinal purposes for more than 2500 years in Ayurvedic and Chinese medicine. Although routinely prescribed in Asian countries for its antimicrobial activity in the treatment of gastrointestinal infections and diarrhoea, and usually used for the treatment of diabetes mellitus, an interest in its beneficial effects in metabolic and cardiovascular diseases has been growing in the Western world over the last decade. Recent literature suggests BBR is a drug with multiple target characteristics, which are already known in traditional medicine. Its activity in carbohydrate and lipid metabolisms, diabetes mellitus treatment, endothelial function and the cardiovascular system has been investigated in the last decade with interesting results both in animals and clinical studies. This review analyzes the scientific literature on the effects and the underlying mechanisms of BBR on carbohydrate and lipid metabolism, endothelial function and the cardiovascular system.

GLUCIDIC METABOLISM

BBR's effects on glucidic metabolism are well known in China, where it has been used as an oral hypoglycemic agent in the treatment of type 2 diabetes mellitus for many years. There are many clinical reports on the hypoglycaemic action of BBR in the Chinese literature, which were confirmed by controlled clinical trials[1].

Several studies have only recently investigated how it may exert its action on glucose metabolism and insulin sensitivity. Insulin resistance is a major metabolic abnormality leading not only to type 2 diabetes, but also to a

group of metabolic disorders known as the metabolic syndrome[2].

In 2006, Lee *et al*[3] investigated the mechanisms underlying the effects of BBR in the treatment of diabetes and obesity and on insulin resistance. Their experiments *in vivo* and *in vitro* paved the way to future understanding. They focused interest on a heterotrimeric protein that plays a key role in the regulation of whole-body energy homeostasis; i.e. adenosine mono-phosphate kinase (AMPK), showing that part of the effects exerted by BBR on diabetes and obesity was due to the stimulation of this protein kinase. The administration of BBR to db/db mice led to a significant body weight reduction with both a significant reduction in fasting blood glucose and improvement in glucose tolerance. Similar effects were also observed in high fat fed Whistar rats, in which BBR administration reduced triglycerides (TG), body weight and improved insulin action in comparison to chow fed rats. The mechanisms that were the basis of these results were detected in the expression of genes involved in energy metabolism. BBR downregulated the expression of genes involved in lipogenesis and upregulated those involved in energy expenditure in adipose tissue and in muscle. Particularly, 11β-hydroxysteroid dehydrogenase, a key enzyme linked to visceral obesity and metabolic syndrome, decreased, and the expression of most genes involved in carbohydrate metabolism was also reduced. In contrast, the transcript level of enzymes related to energy dissipation, including glycerol kinase and acyl-CoA dehydrogenase, increased. These results implied that BBR treatment *in vivo* resulted in a modulation of the gene expression profile that would promote catabolism of high energy intermediates[3].

Others mechanisms involved in BBR actions were clarified by Zhou *et al*[4] and subsequently confirmed by other authors. They reported that BBR promotes glucose uptake in 3T3-L1 preadipocytes through a mechanism distinct from insulin. Insulin increases cellular glucose uptake by promoting GLUT4 expression on the cell surface through the activation of phosphatidylinositol 3-kinase (PI3K). On the contrary, BBR's effect on glucose uptake was insensitive to wortmannin, an inhibitor of PI3K. It seemed that BBR could induce glucose transport by activating GLUT1; particularly BBR increases glucose transport by enhancing GLUT1 gene expression[5]. These effects are mediated by the activation of AMPK, which coordinates both short and long term metabolic changes, leading to an improvement in energy production and a reduction of energy storage. Specifically, its activation results in an increase in the uptake of glucose from the blood to target organs. Further, AMPK inhibits the accumulation of fat by modulating down-stream-signaling components like acetil CoA carbossilase (ACC). AMPK inhibits ACC activity by direct phosphorylation, which leads to a blockage of fatty acid synthesis pathways[6,7]. AMPK phosphorylation and ACC phosphorylation were increased in myoblasts and adipocytes *in vitro* after short-term treatment with BBR, and in liver after long-term BBR treatment of db/db mice[3].

The results of various recent studies have pointed out a possible mechanism of activation of AMPK, mediated by BBR. It was observed that BBR reduced oxygen-dependent glucose oxidation through inhibition of the respirator mitochondrial complex I . To compensate for the reduction in aerobic respiration, it was observed that there was an increase in glycolysis, a biochemical pathway that requires more glucose than aerobic respiration for the production of the same amount of ATP. As a consequence, glucose uptake and its utilization were increased, and associated with a persistent elevation in the AMP/ATP ratio, which induced the activation of AMPK. Also metformin and rosiglitazone, in a dose-dependent manner, inhibited respiration; rosiglitazone displayed similar potency to BBR, while metformin was substantially less potent. These data highlighted the importance of complex I of the mitochondrial respiratory chain as a major target for BBR in order to obtain an activation of AMPK[8,9].

In obese hyperinsulinemic rats, BBR treatment significantly decreased lipid levels, plasma glucose and insulin levels. Oral glucose tolerance tests revealed a decrease of plasma glucose and insulin levels; in addition, the results of insulin tolerance suggested a marked improvement in insulin resistance. According to these *in vivo* results, it was observed that BBR acutely decreased glucose-stimulated insulin secretion in pancreatic β-cells isolated from rats through the AMPK signalling pathway[10].

This evidence was clinically confirmed by a double-blind, placebo-controlled trial, in which BBR administration decreased fasting and postprandial plasma glucose with slightly decreasing postprandial insulin and body weight reduction in type 2 diabetic patients[11].

Another important mechanism underlying the effects of BBR on insulin sensitivity is increases insulin-receptor (InsR) expression in a dose and time-dependent manner. BBR enhancement of InsR expression improves cellular glucose consumption only in the presence of insulin. Silencing the InsR gene reduces this effect. BBR induces InsR gene expression, with a mechanism of transcriptional regulation through protein kinase C (PKC). In type 2 diabetic mice, treatment with BBR lowered fasting blood glucose and fasting serum insulin, increased insulin sensitivity and elevated InsR mRNA, as well as PKC activity in the liver. In addition, it was observed that BBR did not lower blood glucose in type 1 diabetic mice, because of their insulin deficiency[12]. The same results were obtained in a variety of human cell lines and were confirmed in a randomized clinical trial, in which BBR treatment significantly lowered fasting blood glucose, hemoglobin A1c, TG and insulin levels in patients with type 2 diabetes mellitus. In this study, metformin and rosiglitazone were used as references. The effects of BBR on fasting glucose and hemoglobin A1c were similar to those of metformin and rosiglitazone; moreover, BBR showed an important activity in reducing the serum levels of TG. Serum insulin levels declined significantly. Consistent with the *in vitro* experiments, the mean percentage of peripheral blood lymphocytes that express

InsR on the surface, isolated from the patients treated with BBR, was significantly elevated in comparison to that before BBR treatment. These finding confirmed the activity of BBR on the up-regulation of InsR in type 2 diabetes mellitus patients and its relationship with the glucose-lowering effect[13].

Contrary to thiazolidinediones (TZDs), it has been shown that BBR reduces the expression levels of peroxisome proliferator activated receptor γ, suppresses the differentiation of preadipocytes and reduces the accumulation of lipid droplets[14-16]. Thus, unlike TZDs, which may lead to weight gain, BBR may be more suitable for insulin-resistant and diabetic patients with obesity. The insulin-sensitizing and glucose-lowering mechanisms of BBR are of great interest in this field.

In conclusion, we can summarize that, although all the mechanisms underlying BBR's action on glucidic metabolism are not yet completely clarified and continue to be under investigation, its properties, namely reducing fasting blood glucose, hemoglobin A1c, and insulin levels in patients with type 2 diabetes mellitus, reduction of fat mass and TG, improvement of insulin resistance, and reduction of body weight, make BBR a promising molecule for future development in the treatment of glucidic disorders.

LIPID METABOLISM

The main interest of the Western world for BBR properties was initially concentrated on lipid metabolism. In fact, it is an approved nutraceutical substance for treatment of hyperlipidemia in many countries. In 2004, Kong *et al*[17] defined BBR as "a new cholesterol-lowering drug". They demonstrated *in vitro* and *in vivo* the efficacy of this substance in lipid lowering, which was comparable to that of statins. The *in vitro* studies on human hepatocellular liver carcinoma cell lines (HepG2) showed that BBR increases the expression of the liver low-density lipoprotein receptor (LDLR) gene at a post-transcriptional level. The increase was dose and time dependent and was obtained, by stabilizing its mRNA, through the activation of the extracellular signal-regulated kinase pathway. This mechanism is distinct from statins, and indeed this activity is totally independent of intracellular cholesterol levels and has no effects on the activation process of the sterol-regulatory element binding protein (SREBP) or the activity of hydroxymethylglutaryl CoA reductase. These findings translated into clinical results, and, in fact, BBR administration in hypercholesterolemic patients led to a significant cholesterol reduction, which was also evident in animal studies[17]. The same research group subsequently elucidated the mechanism observed by Brusq *et al*[18]. This latter group demonstrated that BBR *via* AMPK activation inhibited cholesterol and TG synthesis in hepatic cells. He assumed that LDLR upregulation, AMPK activation and lipid synthesis inhibition were abolished when the MAPK/extracellular signal-regulated kinase (ERK) pathway was blocked, but he did not identify the exact mechanisms[18]. Some years later, Abidi *et al*[19] clarified that the BBR-induced stabilization of LDLR mRNA is mediated by the ERK signalling pathway through interactions of the cis-regulatory sequences of the 3-untranslated region of the LDLR mRNA and mRNA binding proteins that are downstream effectors of this signalling cascade[19]. Moreover, they identified the LDLR mRNA untranslated region responsible for the rapid mRNA turnover, as the target of BBR action leading to LDLR-mRNA stabilization[20].

An additional mechanism acting on the pro-protein convertase subtilisin/kexin type 9 (PCSK9) has only been recently clarified. PCSK9 downregulates, post-trascriptionally, LDLR by shuttling it to the lysosomes for degradation, thus increasing the level of circulating LDL-cholesterol (LDLc). BBR decreases PCSK9 mRNA and protein levels in a type and dose dependent manner, likely due to a decreased transcription of the PCSK9 gene[21]. Thus, BBR could have dual actions on LDLR metabolism by prolonging its mRNA half-life as well as directly increasing protein abundance through the blockage of PCSK9-mediated degradation. Differently from BBR, statins induce PCSK9 transcription[22], which makes BBR an attractive candidate for enhancing statin efficacy. The mechanisms underlying the transcriptional suppression of PCSK9 by BBR have been recently clarified in an *in vitro* study on HepG2 cells. Li *et al*[23] identified a highly conserved hepatocyte nuclear factor 1 (HNF1) binding site as a critical sequence motif for PCSK9 transcription. BBR reduction of HNF1 and nuclear SREBP2 led to a strong suppression of PCSK9.

This observed synergy would be beneficial with regard to LDLR expression, because SREBP2 is absolutely required for LDLR transcription, and a strong inhibition of SREBP2 would eventually shut down LDLR expression. The fact that BBR treatment increases LDLR protein levels in HepG2 cells, and in livers of hyperlipidemic hamsters *in vivo*, suggests that the balanced effects of BBR are in favour of LDLR expression and stability[12,17].

Since BBR counteracts the inducing effects of statins on PCSK9 transcription, their combination may translate into a synergistic efficacy. In fact the combination of BBR with simvastatin (SIMVA) increased LDLR gene expression to a level significantly higher than that in monotherapies. The rats treated with a combination of BBR and SIMVA showed a significantly reduced serum LDLc by 46.2%, which was more effective than that of the SIMVA (28.3%) or BBR (26.8%) monotherapy. More effective reduction of serum TG was also achieved with the combination as compared with either monotherapy. In addition, a significant reduction of liver fat storage was found after combination therapy[24]. These observations translated into clinical results; in fact, the evaluation of the therapeutic efficacy of the combination in 63 hypercholesterolemic patients showed an improved lipid-lowering effect, with 31.8% reduction of serum LDLc compared with 23.8% with BBR and 14.3% with SIMVA monotherapies. Similar effects were observed in the reduction of total cholesterol as well as TG[24]. What is really intriguing in all these mechanisms is the activa-

tion of AMPK, which has been proposed to play a key role in the regulation not only of glucidic metabolism, as above clarified, but also of lipid one[12]. As observed for LDLR up-regulation, BBR effects on lipid synthesis are mediated by the ERK pathway. AMPK phosphorylates and inactivates ACC, a key enzyme involved in fatty acid synthesis, leading to an increase in fatty acid oxidation, decrease in fatty acid synthesis and TG synthesis inhibition. This finding could be more interesting because AMPK activation has been proposed as a valuable approach to target lipid disorders[25] and because antidiabetic drugs, such as rosiglitazone and metformin, have been described to act, at least partially, through AMPK activation[26].

A more recent study about combination strategy investigated the efficacy in lipid lowering of BBR combined with other nutraceutical compounds. *In vivo* study in hamsters showed that combination with plant sterol improves cholesterol lowering efficacy through a synergistic action on cholesterol absorption in addition to synergistically reducing plasma TG[27]. Some clinical trials confirmed the effectiveness of a combination strategy. Cicero *et al*[28] demonstrated not only the efficacy of BBR alone but also its enhanced potency when combined with other recognized hypolipidemic nutraceutical agents, namely policosanols and red yeast rice (RYR). The efficacy of the same nutraceutical combination in dislipidemic patients has been confirmed recently by Affuso *et al*[29] with a double blind placebo controlled clinical trial, in which a significant reduction of total LDLc and TG was shown in a group of patients with hypercholesterolemia. The intriguing mechanisms involved in glucidic and lipidic metabolism support BBR efficacy in treating metabolic disorders. The sharing of metabolic pathways and the increasing prevalence of metabolic syndrome have focused interest on this molecule. The safety profile demonstrated in the clinical studies and the favourable results in combination therapy support its use not only in mild hyperlipidemia but also in patients that do not tolerate statins or do not achieve therapeutic goals[30] with single therapy.

EFFECTS ON ENDOTHELIUM AND THE HEART

Endothelial dysfunction is an important early event in the pathogenesis of atherosclerosis. The mechanisms underlying endothelial injury are numerous and linked to metabolic alteration. In obesity and insulin resistance, the increased secretion of proinflammatory cytokines and decreased secretion of adiponectine, the increased circulating levels of free fatty acids, and hyperglycaemia may alter gene expression and cell signalling in the vascular endothelium, contributing to changes in the release of endothelium derived factors. Dysfunctional endothelium is characterized by activation of NADPH oxidase, uncoupling of endothelial nitric oxide synthase (eNOS), increased expression of endothelin-1, an imbalance between the production of vasodilators and vasoconstrictor mediators, and induction of adhesion molecules[31]. The altered endothelial homeostasis, in turn, contributes to plaque initiation and progression. It is associated with most cardiovascular disease, such as hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes and chronic renal failure[32]. Endothelial cells exposed to hypercholesterolemia show a reduced capacity to release endothelium-derived relaxing factors, because of LDLc promotion of endothelial eNOS downregulation[33]. Lowering cholesterol levels appears to improve endothelial function[34]. In diabetes and insulin resistance, other mechanisms may trigger endothelial dysfunction. Insulin signalling is altered in these two conditions, and affects the pathway leading to phosphorylation and activation of eNOS, which is also, in this case, dramatically downregulated[35]. eNOS represents a major weapon of endothelial cells to fight vascular disease. It generates nitric oxide (NO), whose role is to dilate blood vessels and maintain vascular homeostasis by stimulating cGMP[36]. Several studies have suggested a central role of endothelial AMPK in maintaining physiological functions, such as mediation of eNOS activation in response to shear stress[37], modulation of endothelial cell energy supply[38], protection from apoptosis[39] and regulation of inflammation, angiogenesis, and maintenance of perfusion[40,41]. Impairment of endothelium dependent relaxation (EDR) represents reduced eNOS derived NO bioavailability, and is the first step in endothelial injury. It is present also in the absence of vessel damage. In 2000, Ko *et al*[42], by *in vitro* investigation, demonstrated that BBR has not only vasorelaxant but also antiproliferative effects. According to their results, BBR could act both on the endothelium and on the underlying vascular smooth muscle cells to induce relaxation (Figure 1). NO, is likely involved in the EDR. More recently, this mechanism has been clarified in endothelial cells isolated from rat. It was confirmed that the vasodilatant effect of BBR was mediated by eNOS leading to NO production through activation of the AMPK cascade. Moreover, BBR counteracts several adverse effects

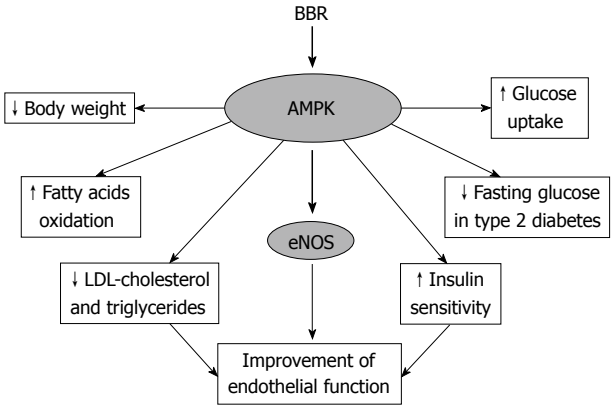

Figure 1 Counteracting effects of Berberine (BBR) on endothelial dysfunction. eNOS: Endothelial nitric oxide synthase; AMPK: Adenosine monophosphate kinase.

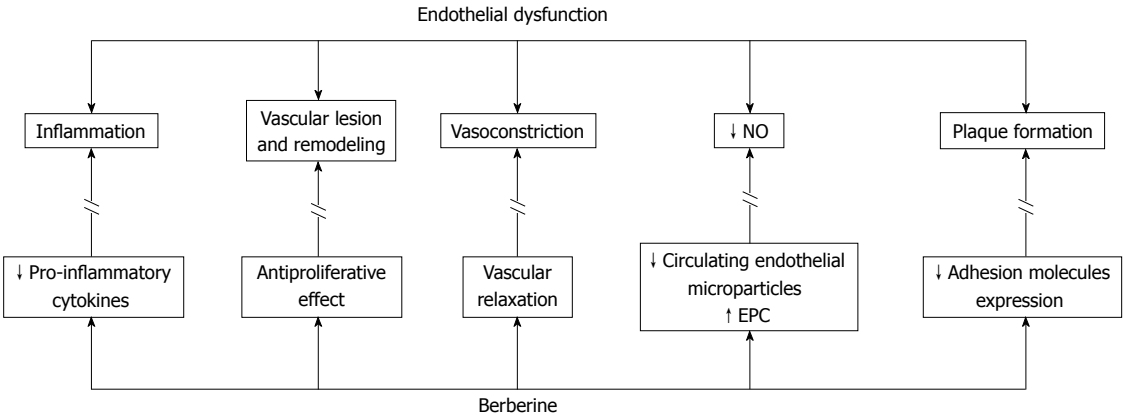

Figure 2 Most metabolic and vascular effects exerted by BBR are mediated by the activation of the AMPK cascade.

of hyperglycemia on the endothelium, including the inhibition of high glucose-induced reactive species intracellular accumulation and cellular apoptosis and inflammation, which characterize vascular injury[31,43]. Another recognized effect of BBR is the significant decrease in the number of adherent monocytes on endothelial cells, which is a key event in the early stages of atherosclerosis. Furthermore, BBR suppresses the activation of the nuclear factor-κB (NF-κB), the expression of adhesion molecules (VCAM-1 and ICAM-1) induced by hyperglycemia and the high glucose-induced elevation of several pro-inflammatory cytokines and chemokines, including tumor necrosis factor-α, IL1-β, IL8 and MCP1, which are other targets of NF-κB involved in the development of atherosclerotic plaques[43] (Figure 1).

Others mechanisms as the basis of BBR effects on the endothelium have been clarified by Xu *et al*[44]. Recently they demonstrated, in healthy subjects, that BBR induces an up regulation of endothelial progenitor cells (EPC-CFUs) through NO production. The number of EPC-CFUs was increased after BBR treatment and the functions, particularly proliferation, adhesion and migration, were enhanced. In addition, the same research group observed that the circulating endothelial microparticles (EMPs), usually associated with endothelial dysfunction, declined after BBR therapy (Figure 2)[45]. This observation was associated with an improvement of flow mediated vasoldilation (FMD) in healthy subjects, underlying a strong relationship with EMPs decline. Moreover the EMPs led to diminished eNOS protein expression *in vitro*, a detrimental effect inhibited by BBR[45]. The clinical effects of BBR treatment on FMD have also been recently demonstrated in a clinical, double blind placebo controlled study, in which BBR was administered combined together with policosanols and RYR. This study demonstrated that the treatment produced a significant improvement of FMD in a population of hypercholesterolemic subjects[29].

Besides the effects on endothelial function, several animal and clinical studies have demonstrated the therapeutic potential of BBR as supportive in the treatment of hypertension, atherosclerosis and heart disease, including left ventricular remodelling[44,46,47]. Specifically, BBR seems to have an inhibitory effect on cardiac hypertrophy, experimentally induced. Hong *et al*[48] demonstrated, in Sprague-Dawley rats with a supra-renal abdominal aorta constriction, that 8 wk of treatment with BBR led to cardiac growth inhibition. Both whole heart and left ventricular weight were notably decreased, and the parameters of cardiac contractility and relaxation improved. Subsequently they investigated the effects of BBR on catecholamine levels, in rats with experimental cardiac hypertrophy, demonstrating that BBR decreased plasma noradrenaline levels and the adrenaline levels both in plasma and in left ventricular tissue[49]. These findings showed the efficacy of BBR in modulating the sympathetic nervous activity of rats with experimental cardiac hypertrophy, and may support the therapeutic potentials of BBR in patients with cardiac hypertrophy and chronic heart failure. In fact, Zeng *et al*[46] investigated the efficacy and safety of BBR administration in patients with congestive heart failure (CHF) secondary to ischemic or idiopathic dilated cardiomyopathy. The addition of BBR to standard therapy showed a significant improvement of left ventricular function, exercise capacity and dyspnea-fatigue index in patients treated with BBR compared with a control group. These positive effects on heart function and symptoms were also associated with an important anti-arrhythmic action. During long-term follow-up, total mortality was significantly lower in the BBR treated patients than in the placebo group. This was due to an apparent decrease in both sudden cardiac death and death due to CHF[46].

The antiarrhythmic effect of BBR and its metabolites (tetra-hydro-berberin and 8-oxo-berberin) is due to the modulation of multiple ion channels both in sarcolemma and the sarcoplasmatic reticulum. Complex mechanisms as the basis of the antiarrhythmic activity have been demonstrated *in vivo* in several animal models[50]. These observations make BBR a promising antiarrhythmic agent with the potential to prevent sudden cardiac death.

CONCLUSION

Although BBR is usually thought of as a traditional Chi-

nese medicine, recent discoveries have provided novel evidence that it may be considered a promising tool to counteract metabolic and cardiovascular (CV) disorders. Particularly, its demonstrated effects on the AMPK cascade, involved in CV disorders and metabolic pathways, propose BBR as a new therapeutic agent in the treatment of type 2 diabetes and metabolic syndrome (Figure 2). In fact, widely used drugs, including statins, metformin and rosiglitazone, execute CV protective effects though the activation of AMPK. The demonstrated efficacy in dyslipidemia and the promising antiarrhythmic activity make BBR a candidate for further study to provide new insights for therapeutic purpose.

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REVIEW

Percutaneous coronary intervention for unprotected left main coronary artery stenosis

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Abstract

Hemodynamically significant left main coronary artery stenosis (LMCA) is found in around 4% of diagnostic coronary angiograms and is known as unprotected LMCA stenosis if the left coronary artery and left circumflex artery has no previous patent grafts. Previous randomized studies have demonstrated a significant reduction in mortality when revascularization by coronary artery bypass graft (CABG) surgery was undertaken compared with medical treatment. Therefore, current practice guidelines do not recommend percutaneous coronary intervention (PCI) for such a lesion because of the proven benefit of surgery and high rates of restenosis with the use of bare metal stents. However, with the advent of drug-eluting stents (DES), the long term outcomes of PCI with DES to treat unprotected LMCA stenoses have been acceptable. Therefore, apart from the current guidelines, PCI for treatment of unprotected LMCA stenosis is often undertaken in individuals who are at a very high risk of CABG or refuse to undergo a sternotomy. Future randomized studies comparing CABG *vs* PCI using DES for treatment of unprotected LMCA stenosis would be a great advance in clinical knowledge for the adoption of appropriate treatment.

INTRODUCTION

Because of the long-term benefit of coronary artery bypass graft (CABG) surgery with reference to medical therapy, CABG has been the standard treatment for unprotected left main coronary artery (LMCA) stenosis[1-3]. However, with the advancement of techniques and equipment, the percutaneous interventional approach for implantation of coronary stents has been shown to be feasible for patients with unprotected LMCA stenosis[3]. In particular, the recent introduction of drug-eluting stents (DES), together with advances in periprocedural and postprocedural adjunctive pharmacotherapies, have improved the outcome of percutaneous coronary interventions (PCI) for these complex coronary lesions[4-29]. Nonetheless, PCI for unprotected LMCA stenosis is still indicated for patients at high surgical risk or with emergent clinical situations, such as bailout procedure or acute myocardial infarction (MI), as an alternative therapy to CABG, because a recent randomized study failed to prove superiority or at least non-inferiority of DES placement for unprotected LMCA stenosis compared with CABG[21,30,31]. In contrast, there is a concern about the long-term safety of DES. The incidence of late stent

thrombosis has been reported to be higher with DES compared with bare-metal stent (BMS) implantation[32-36]. Indeed, the United States Food and Drug Administration has warned that the risk of stent thrombosis may outweigh the benefits of DES in off-label use, such as for unprotected LMCA stenosis[37].

Patients with LMCA stenosis have been traditionally classified into two subgroups: protected (a previous patent CABG surgery graft to one or more major branches of the left coronary artery) and unprotected LMCA diseases (without such bypasses). In this review, we evaluated the current outcomes of PCI with DES in a series of research studies conducted across several countries.

DEFINITION OF SIGNIFICANT LMCA STENOSIS

Coronary angiography has been the standard tool to determine the severity of coronary artery disease. Although the traditional cutoff for significant coronary stenosis has been a diameter stenosis of 70% in non-LMCA lesions, this cutoff in LMCA has been a diameter stenosis of 50%. However, because the conventional coronary angiogram is only a lumenogram providing information about lumen diameter but yielding little insight into lesion and plaque characteristics themselves, it has several limitations due to peculiar anatomic and hemodynamic factors. In addition, the LMCA segment is the least reproducible of any coronary segment with the largest reported intraobserver and interobserver variabilities[38-40]. Therefore, intravascular ultrasound (IVUS) is often used to assess the severity of LMCA stenosis.

A decision of significant stenosis at the LMCA necessitating revascularization should be determined by the absolute luminal area, not by the degree of plaque burden or area stenosis. Because of remodeling, a larger plaque burden can exist in the absence of lumen compromise[41]. Abizaid *et al*[42] reported a 1-year follow-up in 122 patients with LMCA. The minimal lumen diameter by IVUS was the most important predictor of cardiac events with a 1-year event rate of 14% in patients with a minimal luminal diameter < 3.0 mm. Fassa *et al*[43] reported that the long-term outcome of patients having LMCA with a minimal lumen area < 7.5 mm^2 without revascularization was considerably worse than those who were revascularized. Jasti *et al*[44] compared fractional flow reserve (FFR) and IVUS in patients with an angiographically ambiguous LMCA stenosis. However, accurate assessment of ostial LMCA is not always possible. Practically, it is important to keep the IVUS catheter coaxial with the LMCA and to disengage the guiding catheter from the ostium so that the guiding catheter is not mistaken for a calcific lesion with a lumen dimension equal to the inner lumen of the guiding catheter. When assessing distal LMCA disease, it is important to begin imaging in the most co-axial branch vessel. Nevertheless, distribution of plaque in the distal LMCA is not always uniform; and it may be necessary to image from more than one branch back into the LMCA.

FFR may play an adjunctive role in determining significant stenosis at the LMCA. FFR is the ratio of the maximal blood flow achievable in a stenotic vessel to the normal maximal flow in the same vessel[45]. A FFR value of < 0.75 is considered a reliable indicator of significant stenosis producing inducible ischemia[46]. In patients with an angiographically equivocal LMCA stenosis, a strategy of revascularization *vs* medical therapy based on an FFR cut-point of 0.75 was associated with an excellent survival and freedom from events for up to 3 years of follow-up[44].

OUTCOMES OF DES

Safety in terms of the risk of death, MI or stent thrombosis

Although there are disputes regarding the long-term safety of DES, the possibility of late or very late thrombosis is still the major factor limiting global use of DES, especially for unprotected LMCA stenosis. Table 1 depicts the results of recent studies demonstrating the outcomes of DES implantation for unprotected LMCA stenosis. It is clear that none of the clinical studies showed a significant increase in the cumulative rates of death or MI following DES implantation for unprotected LMCA, as compared with BMS. In three early pilot studies which compared the outcomes of DES with those of BMS, the incidence of death, MI or stent thrombosis were comparable in the two stent types during the procedure and at follow-up[4-6]. Of interest, in the study by Valgimigli *et al*[6], DES was associated with a significant reduction in both the rate of MI [hazard ratio (HR) = 0.22, $P = 0.006$] and the composite of death or MI (HR = 0.26, $P = 0.004$) compared with BMS. Considering that restenosis can lead to an acute MI in 3.5% to 19.4%, a significant reduction in restenosis achieved by DES might contribute to the better outcome seen with DES. In fact, a previous study suggested that restenosis at the BMS in LMCA could present as late mortality[47]. In addition, more frequent repeat revascularizations to treat BMS restenosis, in which CABG is the standard of care for unprotected LMCA, may also be related to an increase in complications compared with DES. A recent meta-analysis supported the safety of DES which did not increase the risk of death, MI, or stent thrombosis compared with BMS[18]. In this meta-analysis of 1278 patients with unprotected LMCA stenosis, during a median of 10 mo, the mortality rate in DES-based PCI was only 5.5% (3.4% to 7.7%) and was not higher than BMS-based PCI.

Recently, 3 studies assessed the risk of safety outcomes following the use of DES compared with BMS over 2 years[25-27]. After rigorous adjustment using propensity score or the IPTW (inverse-probability-of-treatment weighting) method to avoid selection bias, which was an inherent limitation of the studies, DES was not associated with a long-term increase in death or MI. Of

Table 1 Outcomes of drug-eluting stents for unprotected left main coronary artery stenosis

	Chieffo *et al*[4]		Valgimigli *et al*[6]		Park *et al*[5]		de Lezo *et al*[28]	Price *et al*[11]	Kim *et al*[20]	Meliga *et al*[29]	Mehilli *et al*[48]	
Stent type	SES, PES	BMS	SES, PES	BMS	SES	BMS	SES	SES	SES, PES	SES, PES	PES	SES
Design	Single center study		Single center study		Single center study		Single center study	Single center study	Single center study	Multicenter DELFT study	Multicenter randomized study	
No. of patient	85	64	95	86	102	121	52	50	63	358	302	305
Age (yr)	63	66	64	66	60	58	63	69	67	66	69	69
Ejection fraction (%)	51[a]	57	41	42	60	62	57	NA	50	49	53	54
Acute myocardial infarction (%)	NA	NA	17	20[a]	9.8	6.6	NA	NA	5	8.4	NA	NA
Bifurcation involvement (%)	81[a]	58	65	66	71[a]	43	42	94	54	74	63	63
Two-stent technique (%)	74	NA	40[a]	15	41[a]	18	18	89	17	43	51	49
Initial clinical outcomes	In-hospital		30 d		In-hospital		In-hospital	In-hospital	In-hospital	In-hospital	30 d	
Death (%)	0	0	11	7	0	0	0	0	0	3	1	2
Myocardial infarction (%)	6	8	4	9	7	8	4	8	10	7	4	4
Stent thrombosis (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	NA	0.3	0.7
TVR (%)	0.0	2.0	0.0	2.0	0.0	0.0	0.0	6.0	0.0	0.8	0.3 (TLR)	0.7 (TLR)
Any events, %	NA	NA	15.0	19.0	7.0	8.0	4.0	10.0	10.0	11.0	5.0	4.6
Long-term outcomes (%)	Cumulative		Cumulative		Cumulative		Cumulative	After discharge	Cumulative	3 yr	2 yr	
Mean follow-up (mo)	6	6	17	12	12	12	12	9	11	NA	NA	NA
Death (%)	4	14	14	16	0	0	0	10	5	9	10	9
Myocardial infarction (%)	NA	NA	4[a]	12	7	8	4	2	11	9	5	5
Stent thrombosis (%)	0.1	0.0	NA	NA	0.0	0.0	0.0	0.0	0.2	0.6	0.3	0.7
TVR (%)	19	31	6[a]	12	2[a]	17	2	38	19	14	9 (TLR)	11 (TLR)
Any MACE (%)	NA	NA	24[a]	45	8[a]	26	NA	44	29	32	21	21

[a]$P < 0.05$ between drug-eluting stent (SES and/or PES) *vs* BMS. BMS: Bare metal stent; NA: Not available; MACE: Major adverse cardiac events including death, myocardial infarction, and TVR; PES: Paclitaxel-eluting stent; SES: Sirolimus-eluting stent; TVR: Target vessel revascularization; DELFT: Drug Eluting stent for left main.

interest, Palmerini *et al*[26] showed a survival benefit of DES over 2 years. These studies supported previous pilot studies in that elective PCI with DES for unprotected LMCA stenosis seems to be a safe alternative to CABG.

With regard to the risk of stent thrombosis, in the series of LMCA DES studies, the incidence of stent thrombosis at 1 year ranged from 0% to 4% and was not statistically different from that with BMS[4-6]. Recently, a multicenter study confirmed this finding, where the incidence of definite stent thrombosis at 2 years was only 0.5% in 731 patients treated with DES[19]. In addition, the Drug Eluting stent for left main multicenter study, which included 358 patients undergoing LMCA stenting with DES, reported that the incidence of definite, probable, and possible stent thrombosis was 0.6%, 1.1% and 4.4%, respectively, at 3 years[29]. In recent large multicenter studies for the ISAR-LEFT-MAIN (Intracoronary Stenting and Angiographic Results: Drug-Eluting Stents for Unprotected Coronary Left Main Lesions) study or MAIN-COMPARE (Comparison of Percutaneous Coronary Angioplasty Versus Surgical Revascularization) study, the incidence of definite or probable stent thrombosis was less than 1%[21,48]. However, because these studies were underpowered to completely exclude the possibility of an increased risk of stent thrombosis over this long time period, further research needs to be performed. Previous studies assessing the long-term outcomes of DES for complex lesions showed inhomogeneous outcomes. For example, recent large trials evaluating the safety of DES for complex lesions showed comparable risks of death or MI for the two stent types[49,50]. The recent large NHLBI (National Heart, Lung, and Blood Institute) study in the United States reported that, the off-label use of DES, compared with BMS, for similar indications was associated with a comparable 1-year risk of death and a lower 1-year risk of MI after adjustment[49]. Of interest, a large study of 13 353 patients in Ontario found that the 3-year mortality rate in a propensity-matched population was significantly higher with BMS than with DES[50]. The comparable or lower incidence of death or MI using DES compared with BMS may be due, at least in part, to the off-setting risks of restenosis *vs* stent thrombosis.

Prognostic factors

Several attempts have been made to predict the long-term outcome of complex LMCA intervention. Predictably, periprocedural and long-term mortality strongly depend on the patient's clinical presentation. In the ULTIMA (Unprotected Left Main Trunk Investigation Multicenter Assessment) multicenter study, which included 279 patients treated with BMS, 46% of whom were inoperable or high surgical risk, the in-hospital mortality was 13.7%, and the 1-year incidence of all-cause mortality was 24.2%[51]. On the other hand, in the 32% of patients with low surgical risk (age < 65 years and ejection fraction > 30%), there were no periprocedural deaths and the 1-year mortality was 3.4%. Similarly, in patients with DES implantation, high surgical risk represented by high EuroSCORE or Parsonnet score, was the independent predictor of death or MI[13,52]. Therefore, it is recommended that continued attention should be paid to this procedure in patients at high surgical risk. More recently, the 'SYNTAX (Synergy between PCI with Taxus and Cardiac Surgery score)', which was an angiographic risk stratification model, has been created to predict long-term outcome after coronary revascularization with either PCI or CABG[30]. In the recent SYNTAX study comparing PCI with paclitaxel-eluting stent *vs* CABG for multivessel or LMCA disease, the long-term mortality was significantly associated with the SYNTAX score[30]. Therefore, for patients with high clinical risk profiles or complex lesion morphologies, who are defined using these risk stratification models, the PCI procedures need to be performed by experienced interventionalists with the aid of IVUS, mechanical hemodynamic support, and optimal adjunctive pharmacotherapies, after judicious selection of patients.

Recurrent revascularization

Compared with BMS, DES reduced the incidence of angiographic restenosis and subsequently the need for repeat revascularization in unprotected LMCA stenosis. In early pilot studies, the 1-year incidence of repeat revascularization following DES implantation was 2%-19% as compared with 12%-31% following BMS implantation (Table 1)[4-6]. Fortunately, in a long-term study of 3 years, the incidence of repeat revascularization remained steady without significant observation of the "late catch-up" phenomenon of late restenosis noted after coronary brachytherapy[21]. Recently, two larger studies confirmed the efficacy of DES[25,27]. The risk of target lesion revascularization over 3 years was reduced by 60% with use of DES[27].

The risk of restenosis was significantly influenced by lesion location. DES treatment for ostial and shaft LMCA lesions had a very low incidence of angiographic or clinical restenosis[14]. In a study which included 144 patients with ostial or shaft stenosis in three cardiac centers, angiographic restenosis and target vessel revascularization at 1 year occurred in only 1 (1%) and 2 (1%) patients, respectively. Although, in those studies, the lack of availability of DES sizes larger than 3.5 mm imposed an overdilation strategy to match the LMCA reference diameter, there were no cardiac deaths, MI or stent thrombosis in this study.

In contrast, PCI for LMCA bifurcation has been more challenging although the prevalence was more than 60% in previous studies[4-6,10,21]. However, repeat revascularization was exclusively performed in patients with PCI for bifurcation stenosis[4-6]. A recent study assessing the outcomes of LMCA DES showed that the risk of target vessel revascularization was 6-fold (95% CI: 1.2-29) in bifurcation stenosis compared with non-bifurcation stenosis (13% *vs* 3%)[13]. The risk of bifurcation stenosis was highlighted in a recent study by Price *et al*[11] where the target lesion revascularization rate after sirolimus-eluting stent implantation was 38% (11). In this study, 94% (47/50) of patients had lesions at the bifurcation and 98% underwent serial angiographic follow-up at 3 and/or 9 mo. This discouraging result questioned the efficacy of DES and suggested the need for meticulous surveillance using angiographic follow-up in PCI for LMCA bifurcation stenosis. However, this study was limited by the exclusive use of a complex stenting strategy (two stents in both branches) in 84% of patients, which may have increased the need for repeat revascularization. Although, there is an ongoing debate[53], a recent report proposed that the complex stenting technique might be associated with the high occurrence of restenosis compared with the simple stenting technique[8]. A subgroup analysis of a large Italian study supported this hypothesis that a single stenting strategy for bifurcation LMCA lesions had a comparable long-term outcome to that for non-bifurcation lesions[24]. Taken together, these finding suggest that the simple stenting approach (LMCA to left anterior descending artery with optional treatment in the circumflex artery) is primarily recommended in patients with a relatively patent or diminutive circumflex artery. Furthermore, future stent platforms specifically designed for LMCA bifurcation lesions may provide better scaffolding and more uniform drug delivery to the bifurcation LMCA stenosis.

With regard to the differential benefit of the type of DES used for the prevention of restenosis, the two most widely used DESs, sirolimus- and paclitaxel-eluting stents, were evaluated in previous studies. In an early study, the research trial, which compared these two DESs, a comparable incidence of major adverse cardiac events was shown with 25% in the sirolimus- (55 patients) and 29% in the paclitaxel-eluting stent (55 patients)[12]. The recent ISAR-Left-Main study compared 305 patients receiving sirolimus- and 302 patients receiving paclitaxel-eluting stents in a prospective randomized design[48]. At 1 year, major adverse events occurred in 13.6% of the paclitaxel- and 15.8% of the sirolimus-eluting stent groups with 16.0% and 19.4% of restenosis, respectively (P = NS). The use of a second generation DES is being evaluated in many studies.

Table 2 Features favoring PCI or CABG

	Indications in favor of PCI	Indications in favor of CABG
Absolute	Suitable coronary anatomy for stenting with preserved left ventricular function (≥ 40%)	Patient who refuses PCI
	Patient who refuses surgery	Contraindication to antiplatelet therapy including aspirin, heparin, and thienopyridine (ticlopidine or clopidogrel)
		History of serious allergic reaction to stainless steel, drugs on drug-eluting stents, and contrast agent
		History of known coagulopathy or bleeding diathesis
		Pregnant women
Relative	Lesion restricted to the LMCA ostium or shaft	Complex coronary anatomies at LMCA, unsuitable for stenting (e.g. severe calcification, severe tortuosity, *etc.*)
	Isolated LMCA lesion	Total occlusions at other major epicardial coronary arteries (≥ 2)
	Bail-out procedure (e.g. dissection at the LMCA complicated during angiography or PCI)	Multivessel stenosis except LMCA
	Acute myocardial infarction at the LMCA, in which emergent revascularization is necessary	Decreased left ventricular dysfunction (< 40%)
	Cardiogenic shock due to LMCA stenosis, in which emergent revascularization is necessary	Extensive peripheral vascular disease, in which placement of guiding catheter or intra-aortic balloon pump is not likely to be performed
	Age ≥ 80 yr	In-stent restenosis at the LMCA, in which repeat PCI is not likely to be performed
	Serious co-morbid disease (e.g. chronic lung disease, poor general performance, *etc.*)	
	Limited life expectancy of less than 1 yr	
	Prior CABG	
	Coronary anatomy, unsuitable for CABG (e.g. poor distal run-off)	

CABG: Coronary artery bypass graft surgery; LMCA: Left main coronary artery; PCI: Percutaneous coronary intervention.

COMPARISON WITH CORONARY ARTERY BYPASS SURGERY

It is surprising to note that current guidelines for unprotected LMCA treatment, in which elective PCI for patients who are treatable with bypass surgery is a contraindication, is based mostly on 20-year-old clinical trials[1-3]. These studies demonstrated a definite benefit of survival with CABG in LMCA stenosis compared with medical treatment. However, application of these results to current practice seems inappropriate because surgical technique as well as medical treatment in these studies is outdated by today's standards and no randomization studies between PCI and CABG with enough power have been conducted. The lack of data on the current CABG procedure used in unprotected LMCA stenosis further precludes a theoretical comparison of the two revascularization strategies. Table 2 lists the patient and lesion characteristics favoring PCI or CABG based on current expert opinion and evidence.

Recently, several non-randomized studies comparing the safety and efficacy of DES treatment for unprotected LMCA stenosis, compared with CABG, were published (Table 3). Chieffo *et al*[7] compared retrospectively the outcomes of 107 patients undergoing DES placement with 142 patients undergoing CABG. They showed that DES was associated with a non-significant mortality benefit (odds ratio = 0.331, $P = 0.167$) and a significantly lower incidence of composites of death or MI (0.260, $P = 0.0005$) and death, MI, or cerebrovascular accident (odds ratio = 0.385, $P = 0.01$) at 1-year follow-up. Conversely, CABG was correlated with a lower occurrence of target vessel revascularization (3.6% *vs* 19.6%, $P = 0.0001$). These findings were supported by Lee *et al*[9], in 50 patients with DES placement and 123 patients with CABG. In this study, although the DES group had slightly higher surgical risk, the rate of mortality or MI at 30 d was comparable between the two treatments. At 1-year follow-up, DES patients had a non-significantly better clinical outcome compared with CABG, reflected by overall survival (96% *vs* 85%) and survival freedom from death, MI, target vessel revascularization, or adverse cerebrovascular events (83% *vs* 75%). However, the survival freedom from repeat revascularization at 1 year remained non-significantly higher for CABG compared to the DES (95% *vs* 87%). The results of a recent multicenter study were in agreement with the previous two reports with regard to the safety outcomes[10]. The PCI group treated with BMS or DES (60%) had a similar incidence of death and/or MI, but a higher incidence of target lesion revascularization compared with the CABG group. Similar safety with PCI compared with CABG was observed in older patients (age ≥ 75 years) by Palmerini *et al*[15]. Recently, a randomized study comparing PCI ($n = 52$) *vs* CABG ($n = 53$) was undertaken in 105 patients with unprotected LMCA stenosis[17]. PCI was performed using either BMS (65%) or DES (35%). The primary end point was the change in left ventricular ejection fraction 12 mo after the intervention. A significant increase in ejection fraction was noted only in the PCI group (3.3% ± 6.7% after PCI *vs* 0.5% ± 0.8% after CABG, $P = 0.047$). In contrast, at 1-year after the procedure, repeat revascularization was significantly lower in the CABG group ($n = 5$) than in the PCI group ((*n* =

Table 3 Comparison of drug-eluting stents to coronary artery bypass surgery for unprotected left main coronary artery stenosis

	Chieffo *et al*[7]		Lee *et al*[9]		Palmerini *et al*[15]		Buszman *et al*[17]		Seung *et al*[21]	
Study design	Registry		Registry		Registry		Randomized study		Registry	
Treatment type	PCI with SES, PES	CABG	PCI with SES	CABG	PCI with SES	CABG	PCI with BMS, DES	CABG	PCI with BMS, DES	CABG
No. of patient	107	142	50	123	157	154	52	53	1102	1138
Age (yr)	64	68	72	70	73[a]	69	61	61	62	64
Ejection fraction (%)	52	52	51	52	52	55	54	54	62	60
EuroSCORE or Parsonnet score (Lee)	4.4	4.3	18.0[a]	13.0	6.0[a]	5.0	3.3	3.5	NA	NA
Initial clinical outcomes	In-hospital		30 d		30 d		30 d		NA	
Death (%)	0.0	2.0	2.0	5.0	3.2	4.5	0.0	0.0	NA	NA
Myocardial infarction (%)	9.0	26.0	0.0	2.0	4.5	1.9	1.9	3.8	NA	NA
TVR (%)	0.0	2.0	0.0	1.0	0.6	0.6	1.9	0.0	NA	NA
Any MACE (%)	NA	NA	0.0	8.0	NA	NA	NA	NA	NA	NA
Cerebrovascular accident	0.0	1.4	2.0[a]	17.0	NA	NA	0.0	2.0	NA	NA
Long-term clinical outcomes	Cumulative after discharge		Kaplan-Meier		Cumulative		At 1 yr		Kaplan-Meier at 3 yr for propensity-matched cohort	
Mean follow-up (mo)	12.0	12.0	6.0	6.0	14.0	14.0	NA	NA	33.9	38.4
Death (%)	2.8	6.4	4.0	13.0	13.4	12.3	1.9	7.5	7.9	7.8
Myocardial infarction (%)	0.9	1.4	NA	NA	8.3	4.5	1.9	5.7	NA	NA
TVR (%)	19.6[a]	3.6	7.0	1.0	25.5[a]	2.6	28.8[a]	9.4	12.6	2.6
Cerebrovascular accident (%)	0.9	0.7	NA	NA	NA	NA	0.0	3.8	NA	NA
Any events (%)	NA	NA	11.0	17.0	NA	NA	NA	NA	NA	NA

[a]$P < 0.05$ between PCI *vs* CABG.

15), although the incidence of death or MI was comparable between the two groups. However, this study was underpowered to assess the long-term clinical effectiveness of PCI compared with CABG.

Stronger evidence for the feasibility of PCI as an alternative to CABG comes from a recent large trial, the MAIN-COMPARE study[21]. In this study, data were analyzed from 2240 patients with unprotected LMCA disease treated at 12 medical centers in Korea. Of these, 318 were treated with BMS, 784 were treated with DES, and 1138 underwent CABG. To avoid bias due to the non-randomized study design, a novel adjustment was performed using propensity-score matching in the overall population at separate periods. In the first and second waves, BMS and DES were exclusively used, respectively. The outcome of stenting in the overall population and each wave were compared with those of concurrent CABG. During 3 years of follow-up, patients treated with stenting were nearly 4 times as likely to need a repeat revascularization compared to those who underwent CABG (HR = 4.76, 95% CI: 2.80-8.11). However, the rates of death (HR = 1.18, 95% CI: 0.77-1.80) and the combined rates of death, MI and stroke (HR = 1.10, 95% CI: 0.75-1.62) were not significantly higher with stenting compared with CABG. A similar pattern was also observed in patients treated with DES or BMS. Another interesting finding in this study was that the majority of repeat revascularizations in PCI patients were treated with repeat PCI instead of CABG. Given the fact that the recommendation for CABG for unprotected LMCA disease was based mostly on survival benefit compared with medical treatment, the lack of a statistically significant difference in mortality may support PCI as an alternative option to bypass surgery. In addition, the current recommendation of routine angiographic surveillance at 6-9 mo after PCI for unprotected LMCA stenosis might increase the unnecessary need for repeat revascularization due to 'oculo-stenotic' reflex.

The ultimate proof of the relative values of PCI *vs* CABG for unprotected LMCA stenosis clearly depends on the results of randomized clinical trials comparing the two treatment strategies. The trials involve a number of technical considerations that could significantly alter angioplasty outcomes. The SYNTAX trial compared the outcomes of PCI with paclitaxel-eluting stents *vs* CABG for unprotected LMCA stenosis in a subgroup from the randomized study cohort[30]. As shown in the subset of patients with LMCA disease comprising 348 patients receiving CABG and 357 receiving PCI, PCI (15.8%) demonstrated equivalent 1-year clinical outcomes of major adverse cardiac and cerebrovascular events including death, MI, stroke and repeat revascularization compared with CABG (13.7%, $P = 0.44$). When the patients were stratified according to the vascular involvement, the event rate in the PCI group was numerically higher for patients with 2-vessel (19.8% *vs* 14.4%, $P = 0.29$) and 3-vessel (19.3% *vs* 15.4%, $P = 0.42$) disease. However, the incidence of these events were numerically lower in the PCI group for patients with isolated LMCA (7.1% *vs* 8.5%, $P = 1.0$) or 1-vessel disease (7.5% *vs* 13.2%, $P = 0.27$). Of interest, the higher rate of repeat revascularization with PCI (11.8% *vs* 6.5%, $P = 0.02$) was offset by a higher incidence of stroke with CABG (2.7% *vs* 0.3%, $P = 0.01$). However, it should be noted that the analysis for LMCA disease was not the primary objective analysis but the post-

hoc analysis, which is hypothesis-generating. Therefore, further randomized studies are warranted to provide a definite answer to this question for a specific cohort of patients having unprotected LMCA stenosis. Another randomized study, the PRECOMBAT (Premier of Randomized Comparison of Bypass Surgery *vs* AngioplasTy using Sirolimus-Eluting Stent in Patients with Left Main Coronary Artery Disease) trial, performed in Korea randomized 600 patients with unprotected LMCA to either CABG or PCI with sirolimus-eluting stents. This study has a non-inferiority design with the primary end point of major adverse cardiac and cerebrovascular events at a mean of 2 years. A more global randomized trial, the EXCEL (Evaluation of XIENCE PRIME™ *vs* Coronary Artery Bypass Surgery for Effectiveness of Left Main Revascularization), is also being carried out to compare PCI and CABG for approximately 2500 patients with unprotected LMCA stenosis.

TECHNICAL ISSUES

Stenting techniques

Stenting for ostial or body LMCA lesions seems very simple as the other stenting technique for non-LMCA coronary lesions. For instance, a brief stent expansion is required to obtain optimal stent expansion and to avoid ischemic complications. In ostial LMCA lesions, the coronary stent is generally positioned outside the LMCA for complete lesion coverage of the ostium. Stenting for bifurcation LMCA lesions, however, are technically demanding and should be performed by experienced operators. In general, the selection of an appropriate stenting strategy is dependent on the plaque configuration surrounding the LMCA. However, in spite of recent randomized studies comparing single-stent *vs* two-stent treatment for bifurcation coronary lesions[54,55], the optimal stenting strategy for LMCA bifurcation lesions has not yet been determined. The current consensus is that the two-stent strategy does not have long-term advantages in terms of the incidence of any major cardiac events compared with the single-stent strategy. Therefore, systemic treatment with the two-stent strategy for all LMCA bifurcation lesions, such as T-stenting, Kissing stenting, Crush technique, or Culotte technique is not generally recommended. Instead, a provisional stenting strategy should be considered as the first-line treatment for LMCA bifurcations without significant side branch stenosis.

IVUS

IVUS is considered a useful invasive diagnostic modality in determining anatomical configuration, selecting treatment strategy, and defining optimal stenting outcomes in either the BMS or DES era[56]. Although a retrospective study showed that the clinical impact of IVUS-guided stenting for LMCA with DES did not show a significant clinical long-term benefit compared with the angiography-guided procedure[57], the usefulness of IVUS guided-stenting may not be hampered by this underpowered retrospective study. The information gathered by IVUS may be crucial for the optimal stenting procedure in unprotected LMCA stenosis with excellent intraclass correlation in the measurement of area and plaque[58]. In fact, angiography has a limitation in assessing the true luminal size of the LMCA because this artery is often short and lacks a normal segment for comparison. Therefore, the severity of LMCA stenosis is often underestimated by misinterpretation of the normal segment adjacent to focal stenosis. In addition to the actual assessment of LMCA lesions before a procedure, use of IVUS is very helpful to obtain an adequate expansion of the DES, to prevent stent inapposition, and to achieve full lesion coverage with the DES.

A recent subgroup analysis from the MAIN-COMPARE study reported a very interesting finding in that IVUS-guidance was associated with improved long-term mortality compared with the conventional angiography-guided procedure[23]. With an adjustment using propensity-score matching for 201 matched pairs, there was a strong tendency for a lower risk of 3-year mortality with IVUS-guidance compared with angiography-guidance (6.3% *vs* 13.6%, log-rank $P = 0.063$, HR = 0.54, 95% CI: 0.28-1.03). In particular, for 145 pairs of patients receiving DES, the 3-year incidence of mortality was lower with IVUS-guidance compared with angiography-guidance (4.7% *vs* 16.0%, log-rank $P = 0.048$, HR = 0.39, 95% CI: 0.15-1.02). Of interest, mortality started to diverge 1 year after the procedure. Therefore, in spite of inherent limitations of the non-randomized study design, this study indicated that IVUS-guidance may play a role in reducing very late stent thrombosis and subsequent long-term mortality. In fact, IVUS evaluations of stent underexpansion, incomplete lesion coverage, small stent area, large residual plaque and inapposition have been found to predict stent thrombosis after DES placement[59-63]. Therefore, we strongly recommend the mandatory use of IVUS in PCI for unprotected LMCA.

Debulking atherectomy

In the BMS era, debulking coronary atherectomy before stenting had been used widely in an attempt to reduce restenosis by removal of plaque burden. However, following the introduction of DES, the role of debulking was restricted due to the dramatic benefit of restenosis reduction. One study suggested a viable role for debulking atherectomy even in the DES era for 99 patients with coronary bifurcations[64]. Of interest, debulking in the main branch and side branch for LMCA stenoses allowed single-stenting in 60 of the 63 LMCA bifurcation stenoses. Surprisingly, during the 1-year follow-up, no serious adverse events occurred. This study indicated that debulking may be preferred in LMCA bifurcations in order to aid a provisional single stenting strategy. In addition, debulking still plays a limited role in facilitating stent delivery. Debulking is used to remove plaque in the LMCA which inhibited advancement of the wire into

the left anterior descending artery. Similarly, a rotablator has been used prior to stenting when calcification in the proximal segment prevents stent delivery or the calcified target lesion is not sufficiently dilated. Therefore, although the data is limited, debulking atherectomy or rotablator still have a limited role even in DES treatment to improve lesion compliance.

Hemodynamic support

Patients in an unstable hemodynamic condition need pharmacological- or device-based hemodynamic support during the procedure for LMCA stenosis. Old age, MI, cardiogenic shock and decreased left ventricular ejection fraction are common clinical conditions requiring elective or provisional hemodynamic support. Hemodynamic support devices include the intra-aortic balloon pump, percutaneous hemodynamic support devices, and left ventricular assist devices. The intra-aortic balloon pump has been used most frequently. Although there is no doubt that the provisional use of an intra-aortic balloon pump in patients with hemodynamic compromise is necessary for a successful procedure, from the literature, the planned use of the balloon pump ranges widely. A study recently suggested the role of intra-aortic balloon pump support in 219 elective LMCA interventions[65]. These authors used a prophylactic balloon pump for a broad range of patients with distal LMCA bifurcation lesions, low ejection fraction of < 40%, use of debulking devices, unstable angina, and critical right coronary artery disease. In that study, interestingly, although the patients receiving elective intra-aortic balloon pump support had more complex clinical risk profiles, the rate of procedural complications was lower than in those not receiving intra-aortic balloon pump support (1.4% *vs* 9.3%, $P = 0.032$). Therefore, the elective use of intra-aortic balloon pump support needs to be considered in patients at a high risk with multivessel disease, complex LMCA anatomy, low ejection fraction or unstable presentations. Hopefully, the new support devices, such as TandemHeart (CardiacAssist, Pittsburgh, Pennsylvania, USA) or the Impella Recover LP 2.5 System (Impella CardioSystems, Aachen, Germany) may improve the feasibility of implementation and the complication rate related to these devices.

Antithrombotics

Although the reported incidence of stent thrombosis in DES treatment for LMCA lesions is very low[66], the fear of stent thrombosis remains a major concern and prevents the more generalized use of DES. Therefore, careful administration of antiplatelet agents is very important to prevent the occurrence of stent thrombosis. In fact, premature discontinuation of clopidogrel was strongly associated with stent thrombosis in several studies[32,67]. Therefore, as generally recommended, dual antiplatelet therapy including aspirin and clopidogrel (or ticlopidine) should be maintained for 1 year. If the patients are at high risk, a high loading dose (600 mg) or lifelong administration of clopidogrel needs to be considered. A recent study added the benefit of aggressive use of clopidogrel in the early period after DES implantation[68]. After stopping clopidogrel between 31 and 180 d, the hazard of cardiac death or MI was 4.20 ($P = 0.009$) compared with stopping clopidogrel between 181 and 36 d. Furthermore, in some institutions in Asian countries, the adjunctive administration of cilostazol has been used to reduce thrombotic complications[69].

Aggressive use of antithrombotics should also be considered for complex lesion anatomy or unstable coronary conditions. For example, as shown in previous studies, the use of glycoprotein Ⅱb/Ⅲ inhibitor may play a role in reducing procedure-related thrombotic complications including death or MI[70]. However, the additive role of the glycoprotein Ⅱb/Ⅱa inhibitor, cilostazol, low molecular weight heparin, direct thrombin inhibitor or other new drugs in DES treatment for LMCA lesions needs to be investigated in future research. Until evidence is accumulated, we have to consider an aggressive combination of antithrombotic drugs before, during or after the procedure to avoid thrombotic complications in high risk patients. Although the features of high risk are not well delineated, off-label use of DES, such as in diabetes mellitus, multiple stenting, long DES, chronic renal failure, or presentation with MI, is a good index of a high risk procedure[71].

CONCLUSION

The current studies, although they are limited by the non-randomized study design, small sample size, and short-term follow-up, have demonstrated the promising procedural and mid-term safety and effectiveness of DES compared with BMS or CABG. With these findings, in our opinion, PCI with DES will progressively increase and can be recommended as the reliable alternative to bypass surgery for patients with unprotected LMCA stenosis, especially as the first line-therapy for ostial or shaft stenosis. Although bifurcation stenosis remains challenging using the percutaneous approach, we are still optimistic as further research into novel procedural techniques, new dedicated stent platforms, and optimal pharmacotherapies may improve patient outcome. Furthermore, we hope, with the upcoming results from randomized clinical trials comparing PCI to CABG for unprotected LMCA stenosis, more confidence in the long-term safety, durability, and efficacy of PCI will be accrued in the near future.

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ORIGINAL ARTICLE

Assessment of neovascularization within carotid plaques in patients with ischemic stroke

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Abstract

AIM: To assess neovascularization within human carotid atherosclerotic soft plaques in patients with ischemic stroke.

METHODS: Eighty-one patients with ischemic stroke and 95 patients without stroke who had soft atherosclerotic plaques in the internal carotid artery were studied. The thickest soft plaque in each patient was examined using contrast-enhanced ultrasound. Time-intensity curves were collected from 5 s to 3 min after contrast injection. The neovascularization within the plaques in the internal carotid artery was evaluated using the ACQ software built into the scanner by 2 of the experienced investigators who were blinded to the clinical history of the patients.

RESULTS: Ischemic stroke was present in 7 of 33 patients (21%) with grade Ⅰ plaque, in 14 of 51 patients (28%) with grade Ⅱ plaque, in 26 of 43 patients (61%) with grade Ⅲ plaque, and in 34 of 49 patients (69%) with grade Ⅳ plaque ($P < 0.001$ comparing grade Ⅳ plaque with grade I plaque and with grade Ⅱ plaque and $P = 0.001$ comparing grade Ⅲ plaque with grade Ⅰ plaque and with grade Ⅱ plaque). Analysis of the time intensity curves revealed that patients with ischemic stroke had a significantly higher intensity of enhancement (IE) than those without ischemic stroke ($P < 0.01$). The wash-in time (WT) of plaque was significantly shorter in stroke patients ($P < 0.05$). The sensitivity and specificity for IE in the plaque were 82% and 80%, respectively, and for WT were 68% and 74%, respectively. There was no significant difference in the peak intensity or time to peak between the 2 groups.

CONCLUSION: This study shows that the higher the grade of plaque enhancement, the higher the risk of ischemic stroke. The data suggest that the presence of neovascularization is a marker for unstable plaque.

Key words: Carotid artery plaques; Cerebral infarction; Contrast-enhanced ultrasonography; Ischemic stroke; Neovascularization

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INTRODUCTION

Carotid plaques are frequently found in patients who have suffered a stroke[1-3]. Neovascularization can be identified within the atherosclerotic plaque on endarterectomy specimens. In neovascularization, new vessels sprout from the vasa vasorum[4-6]. The development of neovascularization is considered to be an important phase in the development of plaque[7] and its vulnerability to rupture increases the risk of cerebral emboli[8,9]. Few imaging techniques can be used to detect and quantify this neovascularization[10,11]; for example, Doppler fails to do so because the neovessels are small with very slow flow and lie close to the major flow in the carotid artery lumen.

The purpose of this prospective study was to determine quantitatively whether there is a difference in the neovascular circulation within carotid plaques between patients with and without ischemic stroke using contrast-enhanced ultrasonography (CEUS).

MATERIALS AND METHODS

Patients

Between September 2006 and May 2008, 86 patients with ischemic stroke were recruited in the Neurological Center of the 2nd Affiliated Hospital of Wenzhou Medical College. Ischemic stroke was defined as focal neurological symptoms lasting > 24 h with or without persisting disabilities together with a computerized tomographic scan positive for ischemic lesions, i.e. a discrete (> 10 mm) subcortical or cortical infarct in the anterior or middle cerebral artery territories, in the absence of a cardiac source of embolism (which was excluded on the basis of medical history and transesophageal echocardiography in every patient). For a patient to be included in the study, the stroke had to be recent, defined as not more than 30 d old. All patients had at least one soft plaque in the common carotid artery wall, its bifurcation, or the internal carotid artery, on the side relevant to the infarct.

Patients with ischemic infarction in the basilar artery territories were excluded as were those with transient ischemic attacks, amaurosis fugax and hemorrhagic strokes. Patients with bilateral hemispheric symptoms and those with known cardiac mural thrombus and patent foramen ovale, (as verified on transesophageal echocardiography) were also excluded from the analysis because of suspected cardioembolic origin.

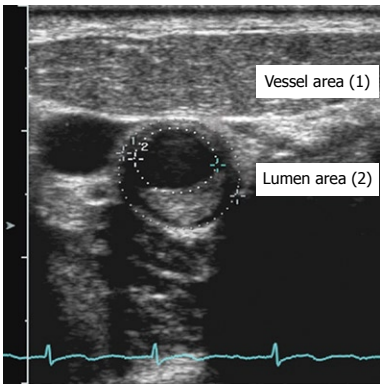

Figure 1 An example of carotid artery atherosclerosis seen with ultrasonography. Percent vessel area stenosis is plaque area (vessel area minus luminal area) divided by vessel area × 100.

Controls

Ninety-seven controls with soft carotid plaques were recruited from 1556 patients referred for thyroid ultrasound examination at the same hospital. History of stroke or other cardiovascular disease was assessed by the investigators. Individuals reporting a positive history of stroke were not eligible, whereas those reporting a positive cardiovascular history other than stroke were eligible.

Informed consent was obtained from all patients and controls prior to their examination, and the local ethics committee approved this study (No. 2006-012).

Ultrasonography

Carotid artery ultrasound was performed with an Acuson Sequoia 512 imaging system (Siemens, Mountain View, CA, USA) equipped with a 15L8 linear transducer (frequency: 8-14 MHz). With the subject supine, the extracranial carotid arteries were visualized in longitudinal and transverse sections. The common carotid arteries, carotid bifurcations, and internal carotid arteries were examined for the presence of atherosclerotic plaque which was defined as an intima-media thickness > 1.2 mm[12]. Soft plaque was defined as plaque tissue producing echogenicity less than that of the surrounding adventitia, in the absence of any calcium. The thickest soft plaque on either side (for the controls) and on the relevant side (for the stroke patients) was selected for study. In each case, the transverse view at the point of maximum plaque thickness was selected, and the corresponding cross-sectional outer vessel areas (VA) and lumen areas (LA) were manually traced (Figure 1). The VA was defined as the area within the adventitial border. Plaque area (PA) was calculated as VA-LA, and %PA (PPA) was calculated as (PA/VA) × 100 (%), as previously described by Erbel *et al*[13].

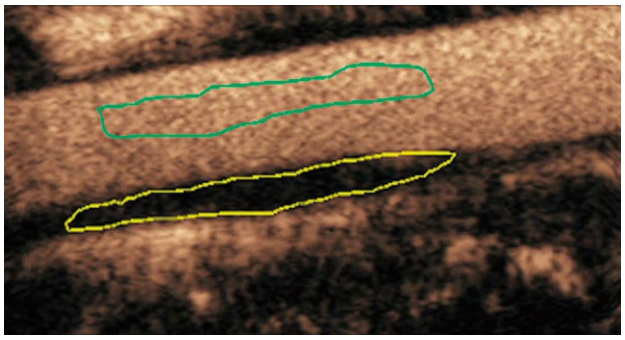

Figure 2 ROI (yellow) was drawn freehand around the peripheral margin of the plaque. The arrival time (AT), time to peak enhancement (TTP), goodness of fit (GOF), baseline intensity (BI) and peak intensity (PI) of plaque were calculated automatically by the ACQ software. IE = PI - BI, WT = TTP - AT.

Contrast-enhanced ultrasound

All subjects were examined with an Acuson Sequoia 512 imaging system equipped with Cadence™ contrast pulse sequencing technology (CPS)[14] by board-registered diagnostic radiologists with a similar amount of experience in vascular sonography (10-16 years). The thickest soft plaque located on the posterior carotid artery wall in each patient and control was examined with real-time ultrasonography using CPS, which uses phase and amplitude modulation to separate the microbubble signals from tissue echoes, at 7 MHz with a low mechanical index (0.35). The microbubble contrast agent SonoVue® (Bracco SpA, Milan, Italy) used in the study was supplied as a lyophilized powder. It was reconstituted by adding 5 mL of saline and gently shaking the vial by hand to form a homogeneous microbubble suspension. The suspension contains 8 μL/mL of sulfur hexafluoride gas stabilized by a phospholipid shell (microbubble concentration 5 mg/mL). The agent was administered intravenously as a 2 s bolus of 2.4 mL through a 19-gauge cannula in an antecubital vein. The cannula was flushed with 10 mL saline. Digital recording of the dynamic sequence was started 5 s after the injection and continued for 3 min.

Data analysis

The digital cine clips were reviewed offline within 3 d of each study by 2 off-site reviewers with 5 years of experience in CEUS who had not been involved in the scanning and were blinded to the clinical data of the stroke patients and controls. The clip loop of each patient was automatically divided into 4 segments, and the first segment (during the arterial phase) was used. The neovascularization within the plaques in the posterior carotid artery was evaluated by visual interpretation and quantitative analysis using the ACQ software. Plaque CEUS enhancement was categorized: grade Ⅰ: non-enhancement; grade Ⅱ: the arterial wall vasa vasorum enhancement; grade Ⅲ: the arterial wall vasa vasorum and plaque shoulder enhancement; and grade Ⅳ: extensive and internal plaque enhancement. For each case, a region-of interest (ROI) was then drawn freehand around the peripheral margin of the plaque using an electronic cursor. Care was taken to exclude the intralumen and periplaque tissues. To allow for the effects of movement due to the patient's breathing, the ROI was adjusted manually frame by frame as necessary. A time-intensity curve (TIC) for the selected plaque tissue and the 4 perfusion parameters for the plaque tissue within the ROI was then derived. The mean values for the four plaque perfusion parameters for each individual patient were then computed by the built-in software (ACQ). These 4 perfusion parameters included arrival time (AT), time-to-peak (TTP), basal intensity (BI) and peak intensity (PI)[14] (Figure 2). Curves with a goodness of fit (GOF) > 0.75 were considered eligible for inclusion. The following normalized indices were calculated manually: The intensity of enhancement (IE) of the plaque was defined as PI minus BI; the wash-in time (WT) was defined as TTP minus AT. All measurements were performed 3 times, and the mean of these 3 measurements was calculated and compared for analysis.

Statistical analysis

Statistical analysis was performed with the Statistical Package for Social Sciences software (SPSS, version 9.0; SPSS, Chicago, IL, USA). Inter- and intra-observer variability for the CEUS quantitative analysis was assessed. The mean differences, standard deviation (SD), and 95% limits of agreement for each of the parameters of EI and WI for each observer and for both observers were calculated using the Bland-Altman test[15]. Intra-observer variability was determined by comparing the first and the second measurements of reviewer 1. Student's *t*-test was used to determine whether there was a significant difference between the stroke patients and controls. Categorical variables including gender, smoking status, and diabetes status were compared between the 2 groups by χ^2 analysis. The percent rate of stroke among different grades of plaque enhancement was also compared by chi-square analysis. A receiver-operating characteristic (ROC) curve was used to calculate the area under the ROC curve (AUC) to determine the grade of plaque enhancement, IE and WT cutoff value with the best sensitivity and specificity. A *P* value of less than 0.05 was considered to indicate a significant difference.

RESULTS

Between September 2006 and May 2008, 128 patients with strokes were referred to the Neurological Center of the 2nd Affiliated Hospital of Wenzhou Medical College. Of these patients, 42 cases were excluded for the following reasons: 26 had hemorrhagic strokes, 12 had ischemic infarction in the basilar artery territory and 4 had bilateral hemispheric symptoms and known cardiac mural thrombus. Among the remaining 86 patients and 97 controls, 5 patients with stroke were excluded because of GOF < 0.75 and 2 controls were excluded because

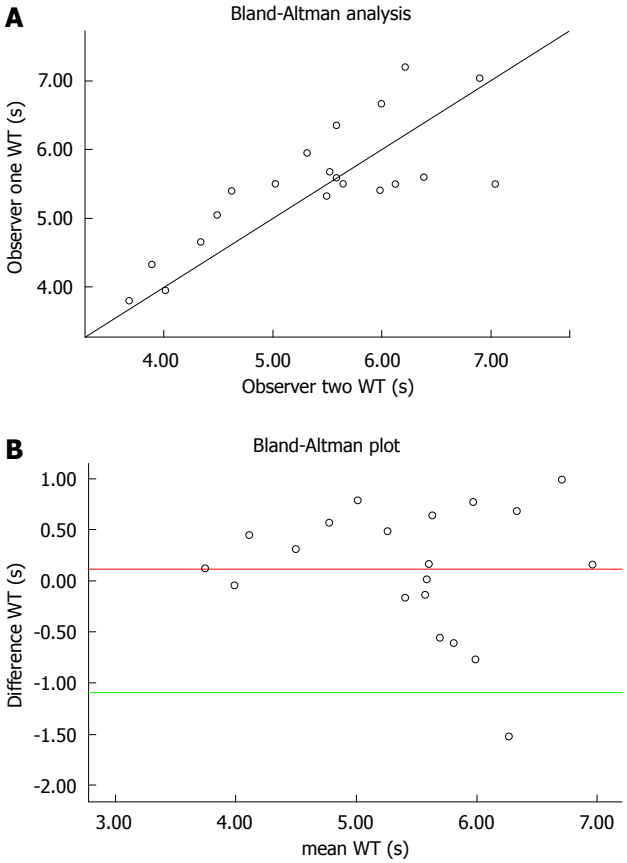

Figure 3 Data for wash-in time (WT) measurements and observer agreement. A: Scatter plot of WT measurements (s) shows data for observer 2 (x-axis) and observer 1 (y-axis); line of perfect agreement is shown; B: Agreement plot for WT measurements made by observers 1 and 2. Plots the difference between observers' measurements and mean measurements. Top and bottom lines show the 95% limits of agreement; middle line shows mean difference.

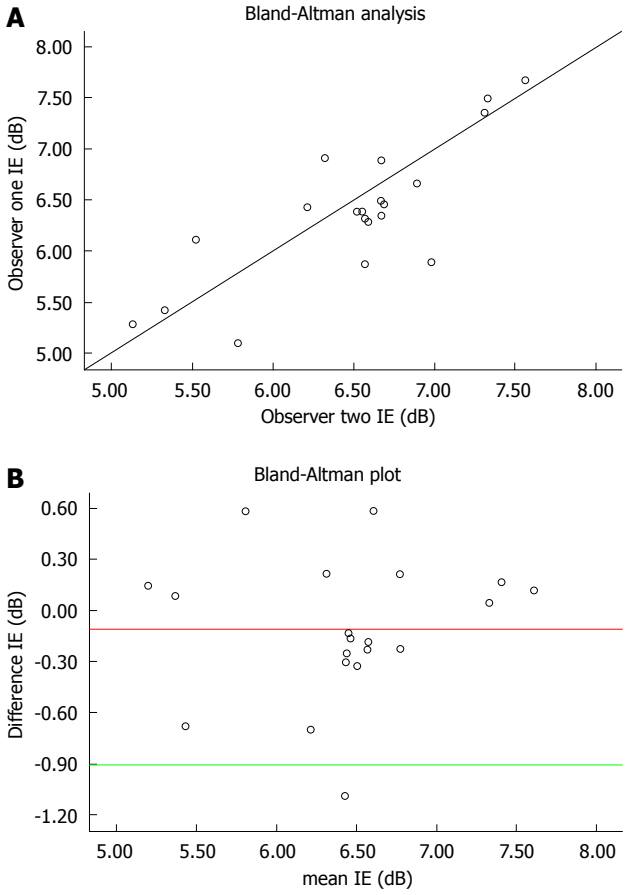

Figure 4 Data for intensity of enhancement (IE) measurements and observer agreement. A: Scatter plot of blood flow measurements (dB) shows data for observer 2 (x-axis) and observer 1 (y-axis); line of perfect agreement is shown; B: Agreement plot for IE measurements made by observers 1 and 2. Plots the difference between observers' measurements and mean measurements. Top and bottom lines show the 95% limits of agreement; middle line shows mean difference.

Table 1 Baseline characteristics of patients with ischemic stroke and controls *n* (%)

Variables	Ischemic stroke (*n* = 81)	Controls (*n* = 95)	*P* value
Age (yr)	62 ± 9	61 ± 12	NS
Men	46 (57)	54 (57)	NS
Women	35 (43)	41 (43)	NS
Systolic blood pressure (mmHg)	146 ± 24	143 ± 22	NS
Diastolic blood pressure (mmHg)	80 ± 15	78 ± 11	NS
Serum total cholesterol (mmol/L)	6.7 ± 1.3	6.5 ± 1.1	NS
Serum HDL cholesterol (mmol/L)	1.4 ± 0.4	1.3 ± 0.4	NS
Diabetes mellitus	6 (7)	7 (7)	NS
Smokers	26 (32)	30 (32)	NS
Percent carotid plaque area	74 ± 4	74 ± 6	NS

HDL: High-density lipoprotein; NS: Not significant.

of incomplete information on data forms. The risk factors and clinical features of the 81 patients with ischemic stroke and 95 controls are shown in Table 1.

Interobserver agreement

The intraclass correlation, mean difference, SD, and 95% limits of agreement for inter-observer measurements for each parameter are summarized in Table 2, with corresponding scatter and Bland-Altman agreement plots for IE and WT in Figures 3 and 4. Similar limits of agreement were obtained between the measurements from the 2 observers. The intraclass correlation ranged from 0.66 to 0.75, indicating good agreement.

Table 2 Inter- and intraobserver agreement for WT and IE

Perfusion measurement	Intraclass correlation	Mean difference	SD	Bland-Altman 95% limits of agreement
WT (s)				
Interobserver	0.75	-0.082	0.61	-1.27 to 1.11
Intraobserver	0.78	0.110	0.62	-1.11 to 1.34
IE (dB)				
Interobserver	0.66	-0.073	0.53	-1.12 to 0.97
Intraobserver	0.81	-0.100	0.41	-0.91 to 0.70

WT: Wash-in time; IE: Intensity of enhancement; SD: 1 standard deviation.

Intraobserver agreement

The intraclass correlation, mean difference, SD, and 95% limits of agreement for interobserver measurements for

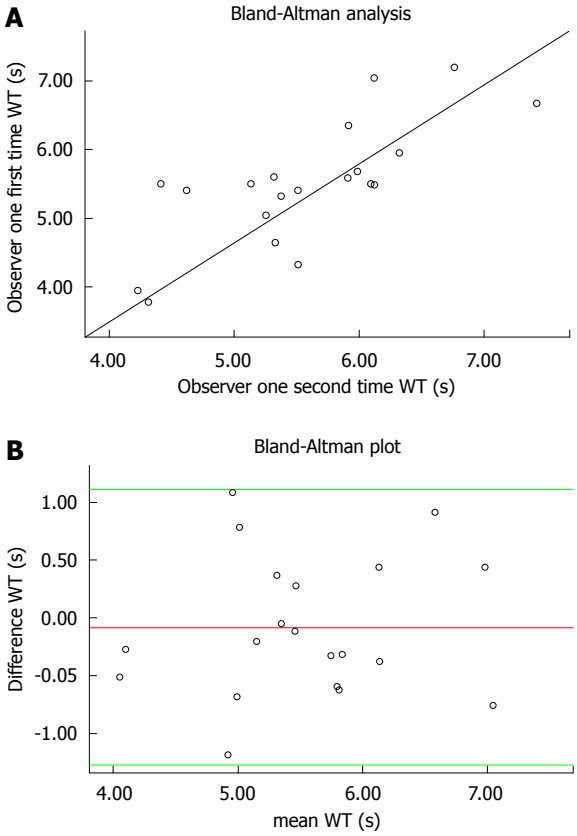

Figure 5 Data for WT measurements and intra-observer agreement. A: Scatterplot of WT measurements (s) shows data for second time (x-axis) and first time (y-axis) measurement of observer 1; line of perfect agreement is shown; B: Agreement plot for WT measurements made by observer 1. Plots the difference between 2 measurements and mean measurements. Top and bottom lines show the 95% limits of agreement; middle line shows mean difference.

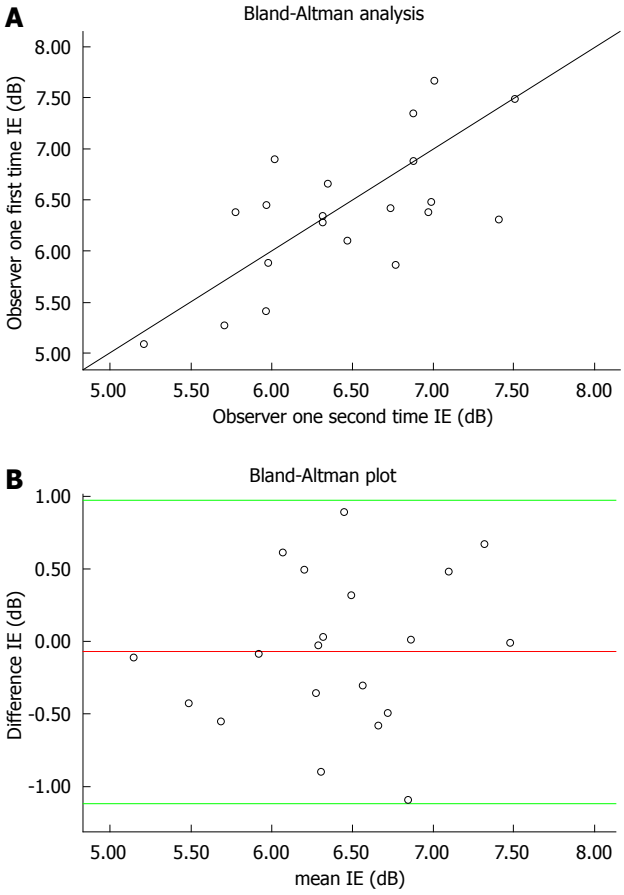

Figure 6 Data for IE measurements and intraobserver agreement. A: Scatterplot of blood flow measurements (dB) shows data for second time (x-axis) and first time (y-axis) measurement of observer 1; line of perfect agreement is shown; B: Agreement plot for IE measurements made by 2 measurements of observer 1. Plots the difference between 2 measurements and mean measurements. Top and bottom lines show the 95% limits of agreement; middle line shows mean difference.

Table 3 Carotid plaque enhancement in patients with ischemic stroke and in controls *n* (%)

Grade of plaque	Ischemic stroke (*n* = 81)	Controls (*n* = 95)	Rate of stroke (%)	*P* value
Ⅰ	7 (9)	26 (27)	21	
Ⅱ	14 (17)	37 (39)	28	
Ⅲ	26 (32)	17 (18)	61	0.001[a]
Ⅳ	34 (42)	15 (16)	69	< 0.001[b]

[a]*P* = 0.001 *vs* grade Ⅰ and Ⅱ; [b]*P* < 0.001 *vs* grade Ⅰ and Ⅱ. Grade Ⅰ: Non-enhancement; Grade Ⅱ: Arterial wall vasa vasorum enhancement; Grade Ⅲ: Arterial wall vasa vasorum and plaque shoulder enhancement; Grade Ⅳ: Extensive and internal plaque enhancement.

each parameter are summarized in Table 2, with corresponding scatter and Bland-Altman agreement plots for IE and WT in Figures 5 and 6. Similar limits of agreement were obtained between the measurements from the 2 reviewers. The intraclass correlation ranged from 0.78 to 0.81, again indicating excellent agreement. Intraobserver agreement was better than interobserver agreement for the WT and IE measurements investigated.

Plaque enhancement was grade Ⅰ in 7 of 81 patients (9%) with stroke and in 26 of 95 controls (27%) (Figure 7A), grade Ⅱ in 14 of 81 patients (17%) with stroke and in 37 of 95 controls (39%) (Figure 7B), grade Ⅲ in 26 of 81 patients (32%) and in 17 of 95 controls (18%) (Figure 7C), and grade Ⅳ in 34 of 81 patients (42%) with stroke and in 15 of 95 controls (16%) (Figure 7D). The rate of stroke in patients with grade Ⅰ plaque was 21%, in patients with grade Ⅱ plaque was 28%, in patients with grade Ⅲ plaque was 61%, and in patients with grade Ⅳ plaque was 69%. The rate of stroke in patients with grade Ⅳ plaque was significantly higher than that in patients with grade Ⅰ or grade Ⅱ plaque ($P < 0.001$) (Table 3). The rate of stroke in patients with grade Ⅲ plaque was significantly higher than that in patients with grade Ⅰ or grade Ⅱ plaque ($P = 0.001$) (Table 3).

The sensitivity and specificity for grade of plaque enhancement (AUC = 0.721, cutoff value > grade Ⅱ) were 74% and 66%, respectively (Figure 8A). Patients who had ischemic stroke had a significantly higher IE than those without ischemic stroke ($P < 0.01$). The WT was significantly shorter in ischemic stroke patients ($P < 0.05$). No other finding was significantly different between the 2 groups (Table 4). The sensitivity and specificity for IE in

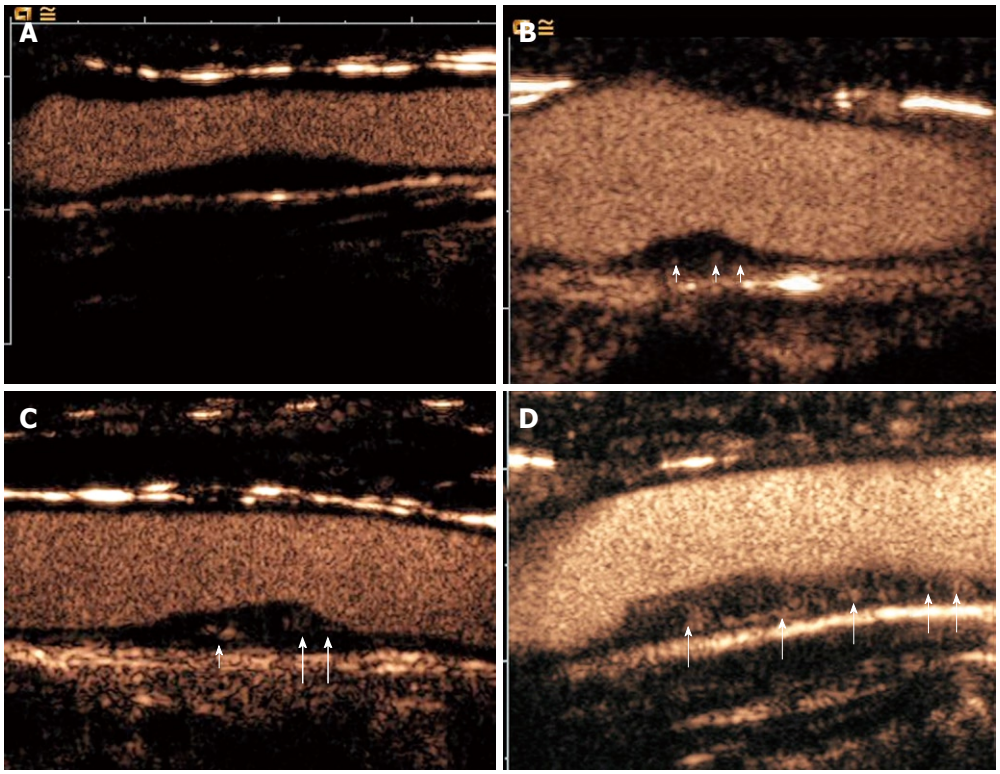

Figure 7 Definition of Grade Ⅰ plaque: non-enhancement of the plaque located on the internal carotid artery. A: Grade Ⅰ: non-enhancement of the plaque located on the internal carotid artery wall; B: Grade Ⅱ: the arterial wall vasa vasorum enhancement (arrows); C: Grade Ⅲ: the arterial wall vasa vasorum (small arrow) and plaque shoulder (large arrows) enhancement; D: Grade Ⅳ: Extensive internal plaque enhancement (arrows).

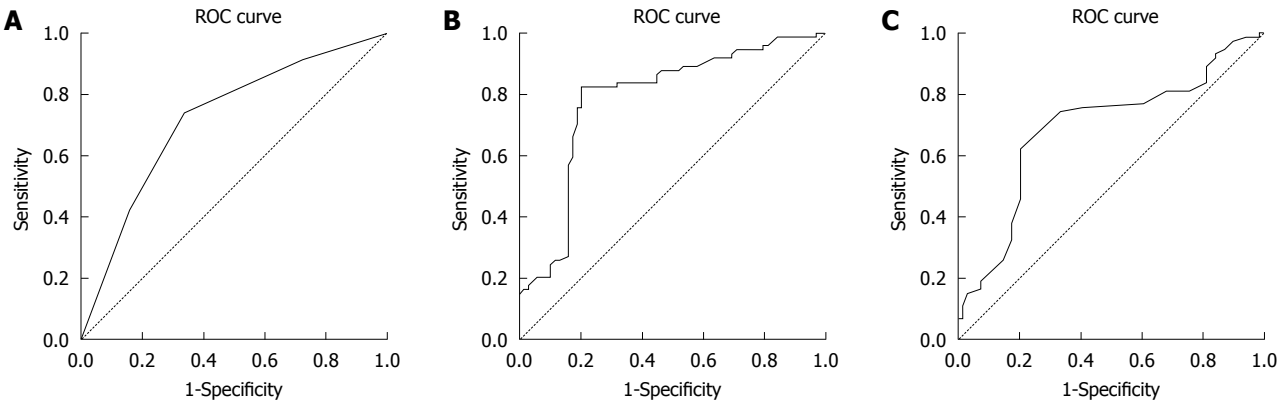

Figure 8 Receiver operating characteristic (ROC) curve of the ability of grade of plaque enhancement to predict precursors of stroke. A: The area under the ROC curve was 0.721. The optimal threshold for the detection of stroke was more than grade Ⅱ, with 74.1% sensitivity and 66.3% specificity; B: Receiver operating characteristic (ROC) curve of the ability of IE to predict precursors of stroke. The area under the ROC curve was 0.785. The optimal threshold for the detection of stroke was 6.4 dB, with 82.4% sensitivity and 79.7% specificity; C: Receiver operating characteristic (ROC) curve of the ability of WT to predict precursors of stroke. The area under the ROC curve was 0.69. The optimal threshold for the detection of stroke was 4.15 s, with 67.6% sensitivity and 73.9% specificity.

the plaque (AUC = 0.785, optimal cutoff value: 6.4 dB) were 82% and 80%, respectively (Figure 8B), and for WT (AUC = 0.690, optimal cutoff value: 4.15 s) were 68% and 74%, respectively (Figure 8C).

DISCUSSION

Recent reports highlight the possibility of demonstrating neovascularization within carotid plaque in real time using CEUS[16,17]. Interest centers on neovascularization as a marker of plaque progression, instability, and rupture[7-9]. This is the first *in vivo* human study of neovascularization within carotid plaques aimed specifically at determining whether there is a difference in neovasculature between patients with and without ischemic strokes.

Using CEUS and calculations based on the ACQ analysis software to quantify the TICs of the CPS signals from the microbubbles, our study revealed that the reproducibility of IE and WT measurements obtained by

Table 4 Contrast-enhanced findings in patients with ischemic stroke and in controls

Variable	Ischemic stroke (n = 74)	Controls (n = 69)	P value
Arrival time (s)	9.6 ± 2.1	9.8 ± 3.4	NS
Time to peak (s)	15.1 ± 3.7	15.6 ± 4.1	NS
WT (s)	5.3 ± 1.4	5.9 ± 1.3	< 0.05
Peak intensity (dB)	8.7 ± 2.7	8.6 ± 3.3	NS
Basal intensity (dB)	2.4 ± 1.0	2.5 ± 1.3	NS
Intensity of enhancement (dB)	6.6 ± 1.4	6.1 ± 1.1	< 0.01

Intensity of enhancement: Peak intensity minus basal intensity.

using CEUS is clinically acceptable for the evaluation of carotid plaque. The intraclass correlation of inter- and intra-observer ranged from 0.66 to 0.75 and 0.78 to 0.81, respectively.

Our study showed that the rate of ischemic stroke in patients with grade Ⅳ or grade Ⅲ plaques was significantly higher than that in patients with grade Ⅰ or grade Ⅱ plaques. The sensitivity and specificity for grade of plaque enhancement (cutoff value > grade Ⅱ) were 74% and 66%, respectively.

Our study also found that patients with ischemic stroke had a significantly greater signal intensity and shorter WT than those without ischemic stroke. The IE in carotid plaques on the relevant side was significantly higher in patients with ischemic stroke than in control patients with plaques but without ischemic stroke (P < 0.01). The WT was also significantly shorter in the ischemic stroke patients (P < 0.05). The sensitivity and specificity for IE in the plaque (cutoff value: 6.4 dB) were 82% and 80%, respectively, and for WT (cutoff value: 4.15 s) were 68% and 74%, respectively. However, we found no significant difference in the PI or TTP between the 2 groups.

The PI measures the maximum IE, and this depends on the BI, which may differ between patients, while the change in intensity after enhancement would be expected to be more robust since it is normalized by the baseline value. The temporal features, AT and TTP are affected by the factors of velocity of bolus injection, patients' height, and cardiovascular status. Therefore, it is not surprising that they were not well correlated with the risk of stroke. This is supported by our finding that the WT, which is corrected for differences in the above factors, was shorter in the ischemic stroke patients, suggesting a more rapid flow in their plaques.

The study by Mofidi *et al*[18] supports our findings. These investigators reported that the presence of neovascularization in the plaque on histology correlated with occlusive clinical events (myocardial infarction and stroke). McCarthy *et al*[19] found a close correlation between the number of plaque neovessels and clinical manifestations in their histological study. Previous studies have reported that gender, race, older age, diabetes mellitus, smoking, and hypertension were predictors of cerebral infarction[20-23]. In our study, we compared all these risk factors and found no significant difference between the groups with and without ischemic stroke.

It is known that the presence of carotid plaques correlates with an increase in the risk of stroke and cerebral infarction[24,25], and that the degree of carotid stenosis is strongly associated with stroke risk in symptomatic patients[26,27]. The parameter of PPA was not significantly different between the 2 groups in our study. Very recently, Coli *et al*[28] reported that carotid plaque contrast-agent enhancement with sonographic agents correlates with the histological density of neovessels and is associated with echo poor plaques (a well-accepted marker of high risk lesions), but is unrelated to the degree of stenosis. Low echo intensity by itself does not correlate with the histological density of vasa vasorum, suggesting that contrast-enhanced ultrasound imaging may identify a subgroup of highly vascularized, potentially vulnerable plaques. Our observation of a positive relationship between contrast-agent enhancement of plaque and ischemic stroke event is in agreement with this report, as higher risk lesions are likely to have a higher value of IE and WT.

Another important finding in our study is that IE and WT were unrelated to plaque thickness. Hyperplasia of vasa vasorum and neovascularization in atherosclerosis may be driven by hypoxia[29] caused by arterial wall thickening, which may be greater in more stenotic lesions, but this does not appear to be the only mechanism. Inflammation and activation of toll-like receptors probably represent another important pathway of promoting angiogenesis in atherosclerotic lesions[30,31].

We only studied the thickest soft plaque in each patient with ischemic stroke and in each control patient, and this might not have been the most culprit plaque. The manual tracking we used to compensate for cardiorespiratory movement has an unknown inaccuracy and could not compensate for out-of-plane movement. Further study is required to evaluate the correlation between the significant dynamic contrast features and histology. Prospective clinical studies are also needed to evaluate the potential impact of contrast-enhanced ultrasound imaging of plaque neovascularization in determining the risk of cerebrovascular events and in monitoring the effect of anti-atherosclerotic therapies.

In conclusion, our study demonstrates that CEUS can be used to quantify the circulation within the neovascularization in carotid atherosclerotic plaques. The higher the grade of plaque enhancement, the higher the risk of ischemic stroke. Patients who had an ischemic stroke on the relevant side had a significantly greater IE and a shorter WT than control patients without stroke, suggesting that the presence of neovascularization is a marker for unstable plaque. The relationships between these findings and the risk of cerebral infarction need further investigation.

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COMMENTS

Background

Carotid plaques are frequently found in patients who have suffered a stroke. Neovascularization can be identified within the atherosclerotic plaque on endarterectomy specimens. The development of neovascularization is an important phase in the development of plaque, and its vulnerability to rupture increases the risk of cerebral emboli. Few imaging techniques can be used to detect and quantify this neovascularization.

Research frontiers

The purpose of the present prospective study was to determine quantitatively whether there is a difference in the neovascular circulation within carotid plaques between patients with and without ischemic stroke using contrast-enhanced ultrasonography (CEUS).

Innovations and breakthroughs

Our study demonstrates that CEUS can be used to quantify the circulation within the neovascularization in carotid atherosclerotic plaques. The higher the grade of plaque enhancement, the higher the risk of ischemic stroke. Patients who had an ischemic stroke on the relevant side had a significantly greater intensity of enhancement and a shorter wash-in time than control patients without stroke, suggesting that the presence of neovascularization is a marker for unstable plaque. The relationships between these findings and the risk of cerebral infarction need further investigation.

Applications

Prospective clinical studies are needed to evaluate the potential impact of CEUS of plaque neovascularization in determining the risk of cerebrovascular events and in monitoring the effect of anti-atherosclerotic therapies.

Peer review

This is an interesting article dealing with the clinical importance of the assessment of neovascularization within carotid plaques in patients with ischemic stroke.

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BRIEF ARTICLE

Impaired diastolic function in naïve untreated human immunodeficiency virus infected patients

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Abstract

AIM: To evaluate cardiac function and structure in untreated human immunodeficiency virus (HIV) patients without clinical evidence of cardiovascular disease.

METHODS: Fifty-three naïve untreated HIV-infected patients and 56 healthy control subjects underwent clinical assessment, electrocardiography (ECG) and echocardiography, including tissue doppler imaging. Moreover, a set of laboratory parameters was obtained from all subjects, including HIV-RNA plasma levels, CD4 cell counts and tumor necrosis factor-α levels.

RESULTS: The two groups showed normal ECG traces and no differences regarding systolic morphologic parameters. In contrast, a higher prevalence of left ventricular diastolic dysfunction (abnormal relaxation or pseudonormal filling pattern) was found in the HIV patients (36% *vs* 9% in patients and controls, respectively, P <0.001).

CONCLUSION: Subclinical cardiac abnormalities appear in an early stage of the HIV infection, independent of antiretroviral therapy. The data suggest that HIV *per se* plays a role in the genesis of diastolic dysfunction.

Key words: Diastolic function; Human immunodeficiency virus; Naïve subjects; Cardiovascular risk factors

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INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) has greatly improved the morbidity and

mortality of human immunodeficiency virus (HIV) patients[1]. However, the spectrum of cardiovascular complications occurring in HIV infection has gradually evolved from opportunistic infections, such as pericarditis, myocarditis and endocarditis, to clinical pictures of atherosclerosis such as myocardial infarction and cerebrovascular events[2,3]. Today, more than 10% of HIV patients experience cardiovascular complications that might have been prevented and require treatment[4].

Cardiovascular complications and metabolic effects of highly active antiretroviral therapy are well known[5-7]. In particular, abnormalities of vascular structure and function[8,9] and echocardiographic patterns of diastolic dysfunction[10] have been frequently reported in HIV patients treated with HAART.

An unresolved issue, however, is whether HIV plays a role in the genesis of the cardiovascular alterations. In a previous paper, we observed a reduction of brachial flow mediated dilation (FMD) and an increase of intima-media thickness (IMT) in naïve HIV patients[11], due to HIV atherogenic effects and/or the associated impairment of the immunological system.

The aim of this study was to establish whether HIV *per se* plays a role in the genesis of cardiac abnormalities reported in HIV infected patients. To this end, we assessed left ventricular morphology and performance in 53 HIV-positive naïve patients without clinical history and/or evidence of cardiovascular disease. Patients were consecutively referred to the infectious disease ambulatory clinic during the last year. Our study also included 56 well-matched healthy control subjects.

MATERIALS AND METHODS

Patients

Fifty-three HIV-positive patients, who had never received antiviral treatment, were consecutively recruited from the ambulatory clinic of the Infectious Disease Department of the University of Naples Federico Ⅱ during the last year, and 56 healthy HIV-negative control subjects recruited from university personnel, matched for age, sex, body mass index, and cigarette smoking characteristics, were enrolled in the study.

Exclusion criteria were: co-infection with hepatitis B or hepatitis C virus, recent opportunistic infection, history of major cardiovascular events (myocardial infarction, transient ischemic attack or stroke), arterial hypertension, use of lipid-lowering agents, glycemia > 100 mg/dL, body mass index > 25 kg/m^2 and renal or liver disease. These criteria were chosen to exclude patients with a history of cardiovascular disease. No patient was taking antihypertensive or antiplatelet drugs. Clinical assessment of the subjects included physical examination and 12-lead electrocardiography (ECG).

All participants gave their informed written consent and the protocol was approved by the University of Naples Federico Ⅱ Ethics Committee.

Analytical methods

Total cholesterol, high-density lipoprotein cholesterol, triglycerides, glucose, homocysteine, and C-reactive protein values were determined using standard laboratory procedures. HIV-RNA plasma levels (copies/mL) were determined by ultrasensitive assay (Roche diagnostics). CD4 cell counts were determined by flow cytometry. Tumor necrosis factor (TNF)-α assays were performed by a chemiluminescent enzyme immunometric method.

Echocardiography

Echocardiography was performed in each subject according to the recommendations of the American Society of Echocardiography[12]. An ultrasound system equipped with a 2.5 MHz multifrequency transducer (Aplio XG imaging system, Toshiba, Japan) was used for complete M-mode, two-dimensional, doppler and tissue doppler imaging (TDI) echocardiographic analyses. Electrocardiographic leads were connected and patients were laid in the left lateral position and examined in standard parasternal long and short axis and apical views. Data were recorded digitally for further blinded offline analysis. Measurements were based on the average of three cardiac cycles. Global intra- and interobserver coefficients of variation were < 7%. A blinded investigator (U.O.) read the echoes offline.

M-mode and two-dimensional measures of left ventricular (LV) architecture were assessed according to standard formulae. The methods are described in detail elsewhere[13]. The following systolic parameters were measured: LV diameters in end diastole and end systole, interventricular septum and LV posterior wall thickness in diastole and systole. LV ejection fractions were assessed using Simpson's biplane rule using conventional apical four- and two-chamber views.

Diastolic function indices were first analyzed by pulsed doppler recordings, with the sample volume located between the tips of the mitral leaflets. The following parameters of diastolic function were measured: peak velocity of early (E wave) and late (A wave) mitral outflow and the E/A ratio, mitral deceleration time (Dct), and the isovolumic relaxation time (IVRT) defined as the time interval between aortic valve closure and mitral valve opening.

Quantitative diastolic data were derived from TDI analysis. The sample volume (4 mm^3) was placed in the left ventricular basal portions of the septal and lateral walls (using the four chambers images) and each parameter was calculated as the mean of both measures. The following parameters were derived: early (E_m) and late (A_m) diastolic velocities and the E_m/A_m ratio. The combined index E/E_m ratio, a method of estimating LV filling pressures[14], was also calculated.

Statistical analysis

The data are expressed as mean ± SD. Comparisons of echocardiographic and TDI parameters between HIV patients and controls were performed by the unpaired

Table 1 Clinical and laboratory parameters of HIV patients and control subjects (mean ± SD)

	HIV patients (n = 53)	Control subjects (n = 56)
Sex (M/F)	36/17	38/18
Age (yr)	38 ± 7	39 ± 8
Body mass index (kg/m^2)	22 ± 3	23 ± 3
Current smoking (%)	38	36
Systolic blood pressure (mmHg)	128 ± 5	120 ± 9
Diastolic blood pressure (mmHg)	78 ± 3	76 ± 7
Total cholesterol (mg/dL)	158 ± 27	160 ± 33
HDL cholesterol (mg/dL)	44 ± 4	45 ± 6
Triglycerides (mg/dL)	127 ± 45	125 ± 40
Glucose (mg/dL)	80 ± 14	82 ± 13
Homocysteine (mg/dL)	11.5 ± 5.1	8.1 ± 4.3
High-sensitivity C reactive protein (mg/L)	0.68 ± 0.79	0.47 ± 0.35

HIV: Human immunodeficiency virus.

Table 2 Viral and immunological parameters of HIV patients and controls (mean ± SD)

	HIV patients (n = 53)	Control subjects (n = 56)
HIV-RNA (copies/mL)	9.754 ± 13.172	
Duration of HIV infection (mo)	29.7 ± 28.0	
CD4+ (cells/mm^3)	370 ± 295	
TNF-α (pg/mL)	18 ± 9[b]	8 ± 6

[b]P < 0.001 *vs* controls. TNF-α: Tumor necrosis factor α.

Student's *t*-test. Pearson's correlation was used when appropriate. Statistical analysis was performed using the SPSS package (SPSS Inc., Chicago, IL).

RESULTS

The two groups (HIV patients and control subjects) were similar in terms of sex, age, and body mass index. Blood pressure levels, metabolic parameters and biomarkers were comparable in the two groups (Table 1). Physical examination did not show any alteration and electrocardiographic data were in the normal range in all subjects.

The viral load, the duration of infection, the immunological data and the cytokine TNF-α mean values are reported in Table 2. The duration from HIV-infection and study enrollment was 29.7 ± 28.0 mo. We did not detect patients who remained untreated for more than 5 years after HIV infection. As shown in Table 2, a significant difference was observed between the TNF-α values in HIV patients and controls (P < 0.001).

The LV ejection fraction was in the normal range both in HIV patients and controls. No differences regarding systolic morphologic parameters were found between the two groups (Table 3).

Data of diastolic function are reported in Table 4. HIV patients with an E/A ratio < 1 were considered to have an abnormal relaxation pattern. Patients with an E/A ratio > 1, and a TDI diastolic parameter E_m/A_m < 1, were considered to have a pseudonormal relaxation pattern. An abnormal relaxation pattern was detected in 16 patients (30%) and 4 controls (7%). Using TDI findings in subjects with an E/A ratio > 1, pseudonormalized flow patterns were observed in 3 more patients (6% of HIV patients) and in 1 control subject (2%). Taken together, 36% of the HIV patients showed an altered diastolic filling pattern (abnormal relaxation or pseudonormal filling) compared with 9% in the control group (P < 0.001).

Among all altered diastolic parameters, the E/A ratio (r = -0.84, Figure 1A) and the combined index E/E_m (r = 0.83, Figure 1B) were strongly correlated with viral loads. We next analyzed the diastolic data in HIV naïve patients in relation to the circulating levels of TNF-α, using a

Table 3 Systolic function in HIV patients and control subjects (mean ± SD)

	HIV patients (n = 53)	Control subjects (n = 56)
M-mode measurements		
LV-ED dimension (mm)	45.6 ± 5.9	46.2 ± 5.1
LV-ES dimension (mm)	31.1 ± 5.9	30.7 ± 6.5
LV-IS diastole (mm)	10.30 ± 2.03	9.90 ± 3.30
LV-IS systole (mm)	13.20 ± 2.85	13.10 ± 3.20
LV-PW diastole (mm)	9.06 ± 2.30	9.30 ± 2.50
LV-PW systole (mm)	12.9 ± 2.7	11.8 ± 3.1
B-mode measurements		
EDV (mL)	107.6 ± 29.9	110.0 ± 33.6
ESV (mL)	44.2 ± 16.5	42.0 ± 17.1
LV-EF (biplane Simpson's) (%)	63.3 ± 7.1	65.1 ± 8.2
SV (mL)	26.0 ± 20.8	24.6 ± 18.9
LV-ET (ms)	308.0 ± 40.8	306.0 ± 41.2

LV-ED: Left ventricular end diastole; LV-ES: Left ventricular end systole; LV-IS: Left ventricular interventricular septum; LV-PW: Left ventricular posterior wall; EDV: End-diastolic volume; ESV: End-systolic volume; LV-EF: Left ventricular ejection fraction; SV: Stroke volume; LV-ET: Left ventricular ejection time.

Table 4 Diastolic function in HIV patients and control subjects (mean ± SD)

	HIV patients (n = 53)	Control subjects (n = 56)	P
Transmitralic Doppler			
Peak E wave velocity (cm/s)	62.7 ± 18.3	79.1 ± 16.4	< 0.001
Peak A wave velocity (cm/s)	57.8 ± 15.7	65.5 ± 21.1	< 0.050
E/A ratio	1.11 ± 0.26	1.25 ± 0.20	< 0.001
Dct (ms)	190.7 ± 24.8	178.1 ± 26.5	< 0.050
IVRT (ms)	98.1 ± 14.3	86.9 ± 14.4	< 0.010
Tissue Doppler			
Peak E_m wave velocity (cm/s)	12.7 ± 8.2	18.9 ± 5.6	< 0.001
Peak A_m wave velocity (cm/s)	10.0 ± 6.9	14.3 ± 4.9	< 0.001
Em/A_m ratio	1.18 ± 0.36	1.36 ± 0.24	< 0.010
Combined index			
E/E_m ratio	6.6 ± 3.5	4.5 ± 1.9	< 0.001

Dct: E wave deceleration time (ms); IVRT: Isovolumetric relaxation time (ms).

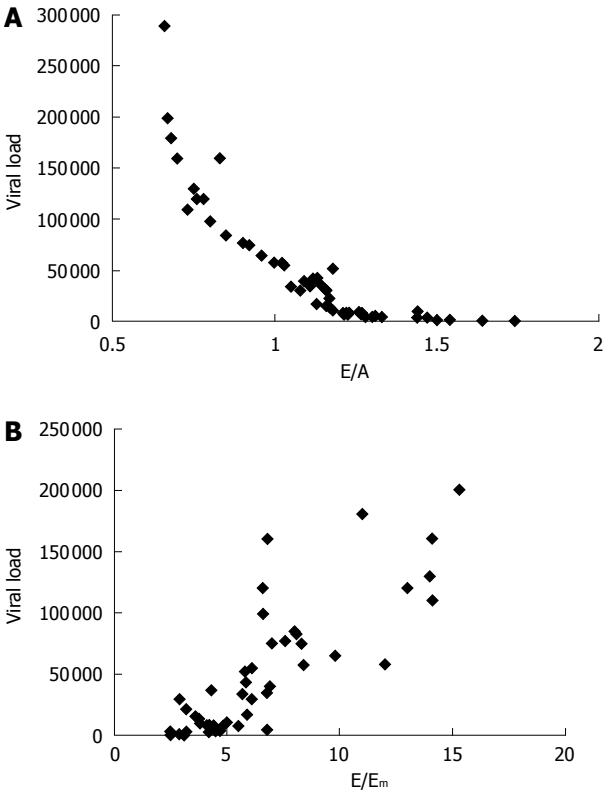

Figure 1 Individual E/A and E/Em values in human immunodeficiency virus (HIV) patients according to viral load (r = -0.84). A: E/A values (r = -0.84); B: E/Em values (r = 0.83).

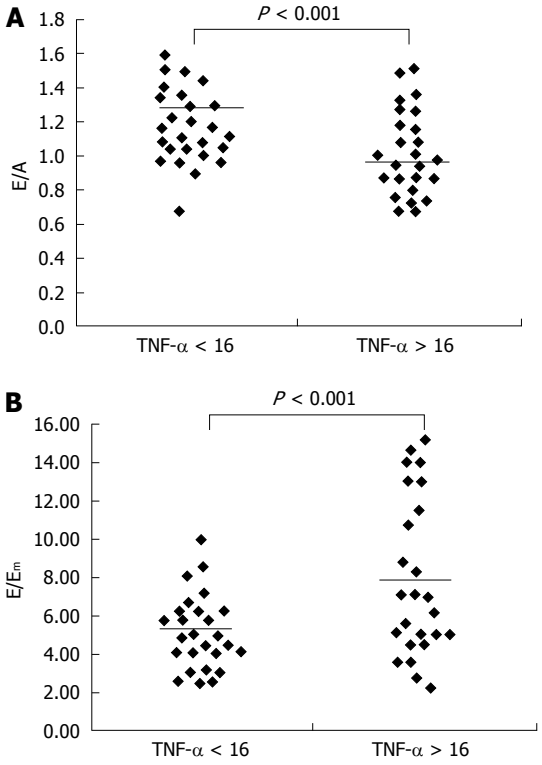

Figure 2 Individual E/A and E/Em ratio values in HIV patients subdivided into two subgroups according to tumor necrosis factor (TNF)-α levels. A: E/A ratio values; B: E/Em ratio values.

cut-off value corresponding to the upper limits of the physiologic range of the cytokine in our healthy population (16 pg/mL). The HIV patients with TNF-α levels > 16 pg/mL showed lower E/A (P < 0.001, Figure 2A) and higher E/Em (P < 0.001, Figure 2B) indexes.

DISCUSSION

In a previous paper, we observed early vascular abnormalities in naïve untreated HIV-infected patients[11].

The aim of this study was to characterize the diastolic function in naïve untreated HIV-infected patients, consecutively referred during the last year to the Ambulatory Clinic of Infectious Disease.

The appearance of abnormalities in diastolic function certainly plays an important role in the increase of the cardiovascular risk in HIV treated patients. Schuster *et al*[15] observed a high prevalence (64%) of diastolic dysfunction in a cohort of HIV-positive patients receiving HAART for > 2 years with no clinical evidence of cardiovascular disease. However, it has proven difficult to distinguish the relative importance of the main potential players; i.e. HIV infection itself, HAART, and the metabolic abnormalities that often cluster with HIV infection.

In this paper, we present the first evidence of diastolic dysfunction in HIV-infected patients who have never received antiretroviral treatment. Because of our exclusion criteria, the metabolic parameters of the HIV patients were within normal ranges and were very similar to the control values. Thus, our results suggest that HIV *per se* can trigger mechanisms that lead to diastolic dysfunction at an early stage of the infection.

HIV may impair diastolic function by inducing abnormalities of both cardiomyocytes and myofibrils with a consequent increase of cardiac stiffness. Indeed, Pozzan *et al*[16] showed cardiac alterations in 83% of untreated AIDS patients' necropsies. In particular, these authors detected cardiomyocyte apoptosis and ultrastructural damage, such as mithocondriosis with increased dense bodies, increased lipofuscin pigment granules, and reduction and disarray of myocardial myofibrils.

Moreover, HIV may determine diastolic dysfunction through the inflammatory and immunological responses caused by the infection and related to the viral load.

In our study, in fact, we observed a clear correlation between both the E/A ratio and E/Em ratio with the HIV-RNA copy number, suggesting that the viral load plays a role in the genesis of cardiac alterations.

Monsuez *et al*[17] reported cardiac involvement with cardiomyocyte apoptosis and an increase of connective trabeculae in the course of HIV infection primarily due to proinflammatory cytokines.

We also detected elevated plasma levels of TNF-α in our HIV infected patients and the levels of TNF-α were directly correlated with plasma HIV-RNA copies. Moreover, the diastolic indexes E/A and E/Em were more

often altered in HIV patients with increased TNF-α values, and high levels of TNF-α have been reported in patients with coronary artery disease who showed diastolic dysfunction but preserved systolic function[18].

In conclusion, our data support the hypothesis that HIV *per se* can play a role in the genesis of diastolic dysfunction detected in our patients from an early stage of the infection.

The major limitation of the study is represented by the absence of structural findings in our "naïve patients", such as histological samples of cardiomyocytes infected by HIV. For these reasons, the relation between the diastolic dysfunction observed in our patients and HIV infection must be considered a strong association, but not a clear cause-effect relationship. Further studies with possible tissue analysis and findings suggestive of HIV infection derived from the cardiac muscle before starting HAART protocols are needed to confirm the hypothesis that HIV is responsible for the genesis of the impairment of the cardiac diastolic indexes.

However, cardiovascular risk assessment and regular cardiovascular evaluation, including echocardiography, should be considered in HIV infected patients from the beginning of the disease. The early detection of cardiovascular involvement might be useful in order to start a program of preventive medicine as soon as possible, which should be instituted even if the clinical relevance of early diastolic dysfunction and the eventual progression to diastolic heart failure or HIV cardiomyopathy have not been completely clarified. This program should primarily include the treatment of coexisting risk factors (e.g. diabetes, hypertension and dislipidemia) and lifestyle interventions, restricting pharmacologic treatments to selected cases with early signs of diastolic heart failure[19].

COMMENTS

Background

Human immunodeficiency virus (HIV) patients treated with the new antiretroviral agents [Highly active antiretroviral therapy (HAART)] showed a major incidence of cardiovascular events as myocardial infarction and ischemic stroke. These complications have been related to the metabolic effects of HAART regimens. However, the authors have often detected early cardiovascular alterations in untreated HIV infected subjects. For this reason they hypothesized that HIV per se can play a role in the genesis of the cardiac abnormalities frequently detected during the course of the infectious disease. To support the authors' hypothesis, they evaluated fifty three naïve untreated HIV patients without clinical history and/or evidence of cardiovascular disease and observed a significant increase of echocardiographic signs of diastolic dysfunction as compared with 56 healthy HIV-negative control subjects recruited from the university personnel.

Research frontiers

HIV plays a role in the genesis of cardiac abnormalities through the inflammatory and immunological responses caused by infection and related to the viral load. The data lend support to the viral infectious theory of atherosclerosis, even if the association between HIV infection and appearance of cardiovascular abnormalities need to be confirmed by further studies with tissue analysis and findings suggestive of HIV infections in cardiac muscle.

Innovations and breakthroughs

The widespread use of the new antiretroviral agents (HAART) has been associated with the major incidence of cardiovascular events detected in HIV treated patients. In this study, the authors present the first evidence of diastolic dysfunction in HIV-infected patients who have never received antiretroviral treatment. Due to the exclusion criteria adopted, the metabolic parameters of the HIV patients were within normal range and very similar to the control values. Thus, the results suggest that HIV per se can trigger mechanisms that lead to diastolic dysfunction at an early stage of the infection.

Applications

Two are the major implications of the study: (1) HIV contributes to the cardiac abnormalities through the inflammatory and immunological responses caused by infection and related to the viral load. Other infections caused by different viruses with the same mechanisms of action might be complicated by the appearance of similar early atherosclerotic lesions; and (2) Echocardiographic assessment should be considered in HIV infected patients from the early stages of the disease for starting a program of preventive medicine as soon as possible.

Peer review

This study showed a possibility that HIV infection per se induces diastolic dysfunction, which may be caused by proinflammatory cytokine-mediated structural damage in the heart. The data were well-presented, and the conclusions were properly conducted. It will be a great article as start off point and it needs further sudies with possible tissue analysis and findings suggestive of HIV infection in the cardiac muscle will be more helpful.

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CASE REPORT

Percutaneous coronary intervention for acute myocardial infarction in a patient with dextrocardia

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Abstract

Situs inversus with dextrocardia is a rare congenital anomaly. There are limited published case reports of successful percutaneous coronary intervention (PCI) in these patients who have atherosclerotic coronary artery disease, especially when presenting with acute myocardial infarction. PCI is technically difficult because of mirror image dextrocardia. We hereby describe a 48-yr-old female, who had acute inferior wall myocardial infarction and underwent successful emergency primary coronary angioplasty and stenting of a proximally occluded right coronary artery. Technical details about PCI are discussed.

Key words: Dextrocardia; Acute myocardial infarction; Percutaneous coronary intervention; Primary angioplasty

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INTRODUCTION

Situs inversus with dextrocardia is a rare congenital malposition of heart and thoraco-abdominal viscera. Coronary artery disease (CAD) with dextrocardia is presumed to be of similar frequency as in the general population. The coronary angiography and percutaneous coronary intervention (PCI) in these patients is technically difficult and requires some modification, such as mirror image angiographic angulation, proper catheter selection and catheter manipulation for selective cannulation of the coronaries. We describe a case of dextrocardia and acute myocardial infarction, with successful emergency coronary angioplasty and stenting of a totally occluded proximal right coronary artery (RCA).

CASE REPORT

A 48-yr-old pre-menopausal hypertensive female presented with acute persistent chest pain of approximately 24 h duration in May 2007. She was a known case of situs inversus with dextrocardia. Clinical examination revealed a right-sided apex beat and no left ventricular third heart sound or murmur. Her routine biochemistry was normal and lipids showed total cholesterol was l84 mg%, HDL was 36 mg%, LDL was 40 mg%, and triglycerides were 101 mg%. ECG showed a negative P wave in the I and aVL limb leads, a positive R wave in the aVR limb leads, a prominent S wave in the left side chest leads and a prominent R wave in the right sided chest leads (Figure 1A and B), which is suggestive of situs inversus with dextrocardia. A ST elevation in the

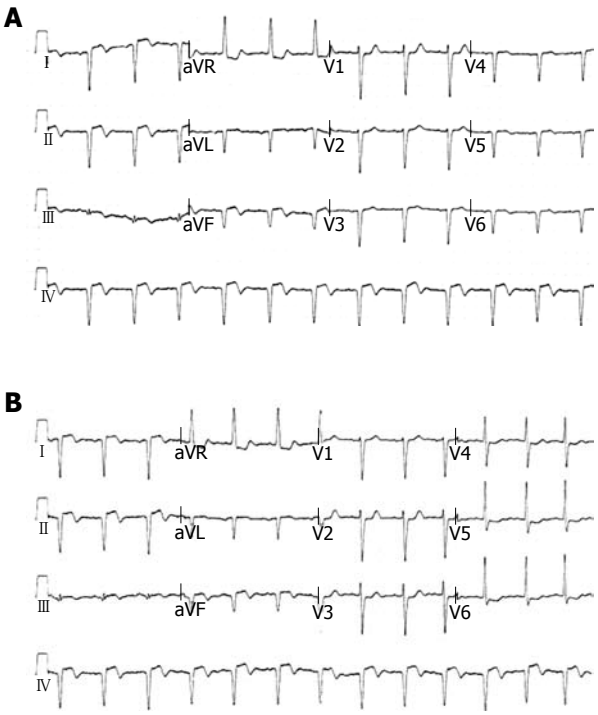

Figure 1 ECG findings in dextrocardia. A: ECG of standard limb and chest leads suggestive of dextrocardia and inferior wall myocardial infarction; B: ECG of standard limb leads and right sided chest leads suggestive of dextrocardia.

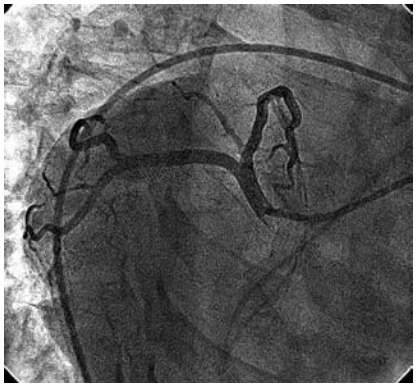

Figure 2 Left coronary angiogram with a JL4, 6F catheter in a RAO 45°, caudal 20° view showing a normal left main artery and its branches.

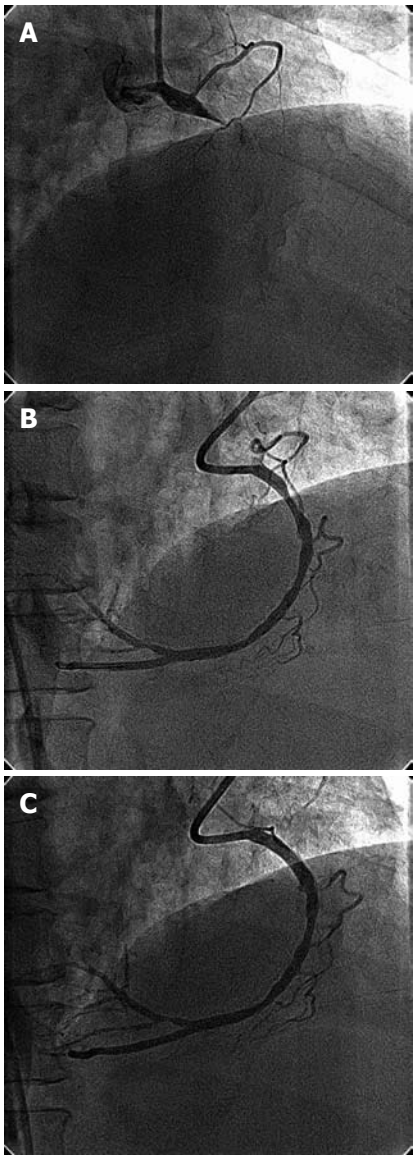

Figure 3 Percutaneous coronary intervention (PCI) of right coronary artery (RCA) in dextrocardia. A: The diagnostic angiogram with a JR 4, 6F catheter in a RAO 40°, cranial 20° view showing total cutoff of the proximal RCA; B: The RCA in a RAO 40°, cranial 20° view showing a proximal lesion following balloon angioplasty; C: The RCA in a RAO 40°, cranial 20° view following stenting showing TIMI-3 flow.

inferior limb leads was suggestive of acute inferior wall myocardial infarction. She was prepared for urgent coronary angiography. The left coronary was easily cannulated with a Judkins Left 4, 6F catheter. The left main coronary artery and its branches were normal (Figure 2). The RCA was cannulated with anti-clockwise rotation of a Judkins Right 4, 6F catheter. There was initial difficulty in localizing the RCA ostium in the aortic root, but later it was selectively cannulated and showed proximal total cutoff (Figure 3A). Following written informed consent for PCI, the RCA was attempted to be selectively cannulated with an extra support guide catheter (ECR 3.5, 6F, Medtronic), but this was not successful despite an anti-clockwise rotation and changing the angulation from LAO 60° to mirror image RAO 60°. During manipulation, the catheter entered twice in the left ventricle and resulted in an episode of ventricular tachycardia each time, which was successfully reverted to normal sinus rhythm by 300 Joules DC shock. Later, a Judkins Right 3.5, 6F guide catheter was able to cannulate the RCA ostium successfully, though with some difficulty and following anti-clockwise rotation. The proximal RCA lesion was crossed with an All Track Wire (ATW) coronary guide wire (Cordis Co., Miami, Florida) and dilated with a 2.5 mm × 20 mm Sprinter balloon (Medtronic, Inc., Minneapolis, Minnesota) thrice, which restored the flow in the RCA (Figure 3B). The door to balloon time was 90 min. Injection of an abciximab intra-coronary bolus was followed by intravenous infusion. A 4 mm × 15 mm Driver stent (Medtronic) was deployed at 15 atmospheres in the proximal RCA, which resulted

in TIMI-3 flow (Figure 3C). The total fluoroscopy time of the procedure was 38 min. A 2-D echocardiogram on the next day showed situs inversus and dextrocardia, mild inferior wall hypokinesia and a left ventricular ejection fraction of 0.60. She had an uneventful recovery and was discharged after 2 d on dual antiplatelets, atorvastatin and anti-hypertensive drugs. At 34 mo of follow-up, she was asymptomatic in NYHA functional class 1.

DISCUSSION

Situs inversus with dextrocardia is a rare congenital anomaly and presents in approximately one per 10 000 individuals[1]. In the absence of other structural heart disease, the life expectancy is usually normal. The association of CAD in these patients is the same as in the general population[2], hence, even elderly patients with this rare anomaly have been subjected to successful percutaneous or surgical intervention of CAD, as reported in the literature[3,4]. The selective cannulation of the RCA might be difficult because of mirror images, for which the operator is not accustomed and also one has to rotate the catheter anticlockwise in the aorta, instead of the routine clockwise rotation for cannulation of the RCA[5].

In the index case, the RCA cannulation was difficult both for a diagnostic angiogram and for PCI, even though it was tried in a standard LAO 60° and also the mirror imaged RAO 60° view, and with anti-clockwise rotation of the catheter. This technical difficulty was secondary to different spatial positions of the RCA ostium, for which one is not accustomed in routine catheterization practice. A double-inversion technique of Goel[6], in which all angiographic pictures are normalized to the standard conventional pictures, as seen in a normally located heart by doing a combination of a right-left reversal of the image on the monitor using the "horizontal sweep reverse" function during acquisition and a reversed RAO/LAO angle selection, can help in better localization and delineation of the coronary anatomy. However, following selective cannulation of the RCA and guide wire crossing, we could perform the successful PCI without the use of the "double reverse technique". Though PCI in dextrocardia patients has already being reported in the published literature[3,7-9], the index case is important in view of the technical difficulty in selective cannulation of the ostium of the RCA and successful emergency primary angioplasty of a totally occluded RCA. In conclusion, we report a rare case of acute myocardial infarction in a case of situs inversus with dextrocardia, with successful emergency primary coronary angioplasty and stenting of a totally occluded RCA.

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Meetings

Events Calendar 2010

January 12-13
Riyadh, Saudi Arabia
1st International Cardiovascular Pharmacotherapy Conference

January 17-21
Hollywood, United States
22nd Annual International Symposium on Endovascular Therapy

January 20-23
Sao Paulo, Brazil
World Cardiology, Metabolism and Thrombosis Congress

January 21-24
Phoenix, United States
13th Society for Cardiovascular Magnetic Resonance Annual Scientific Sessions

January 28-30
Brussels, Belgium
29th Belgian Society of Cardiology Annual Scientific Meeting

January 28-31
Nashville, United States
31st Annual Meeting of The American Academy of Cardiovascular Perfusion

February 3-6
Snowbird, United States
35th Annual Cardiovascular Conference at Snowbird

February 4-5
Leuven, Belgium
Leuven Symposium on Myocardial Velocity and Deformation Imaging

February 6-9
St. Petersburg, United States
10th Annual International Symposium on Congenital Heart Disease

February 8-10
Tel Aviv, Israel
10th International Dead Sea Symposium on Cardiac Arrhythmias and Device Therapy

February 11-12
London, United Kingdom
2nd National Chronic Heart Failure and Hypertension

February 18-21
Istanbul, Turkey
The 2nd World Congress on Controversies in Cardiovascular Disease (C-Care)

February 22-25
Maui, United States
Arrhythmias & the Heart Symposium

February 22-26
Cancun, Mexico
15th Annual Cardiology at Cancun-Advances in Clinical Cardiology and Multi-Modality Imaging

February 25-28
Valencia, Spain
First International Meeting on Cardiac Problems in Pregnancy

February 26-28
Hong Kong, China
International Congress of Cardiology

February 28-March 4
Scottsdale, United States
International Congress XXIII on Endovascular Interventions

February 28-March 5
Keystone, United States
Keystone Symposia: Cardiovascular Development and Repair (X2)

March 3-5
Kish Island, Iran
Islamic Republic of 4th Middle East Cardiovascular Congress

March 4-7
Newport Beach, United States
30th Annual CREF: Cardiothoracic Surgery Symposium

March 7-12
Snowmass Village, United States
Interventional Cardiology 2010: 25th Annual International Symposium

March 14-16
Atlanta, United States
American College of Cardiology 59th Annual Scientific Session

March 18-20
Rome, Italy
VIII Congress of the Italian Society of Cardiovascular Prevention

March 18-20
Prague, Czech Republic
XI International Forum for the Evaluation of Cardiovascular Care

March 24-25
Jeddah, Saudi Arabia
12th KFAFH Cardiovascular Conference: A balanced approach to treatment of cardiovascular diseases

April 8-11
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The 12th South China International Congress of Cardiology

April 14-15
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The 57th Annual Congress of the Israel Heart Society in Association with The Israel Society of Cardiothoracic Surgery

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59th European Society for Cardiovascular Surgery International Congress

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Prague, Czech Republic
EuroPRevent 2010-Cardiovascular Prevention: a Lifelong Challenge

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St. Paul, United States
Controversies in Cardiovascular Disease: Practical Approaches to Complex Problems: Medical and Surgical

May 12-16
Marrakesh, Morocco
7th Metabolic Syndrome, type II Diabetes and Atherosclerosis Congress

May 17-20
Whistler, Canada
6th IAS-Sponsored HDL Workshop on High Density Lipoproteins

May 21-22
Sydney, Australia
3rd Cardiovascular CT, Concord Conference 2010

May 29-June 1
Berlin, Germany
Heart Failure Congress 2010

June 1-4
Seoul, Korea, Republic of
9th Asian-Pacific Congress of Cardiovascular & Interventional Radiology (APCCVIR 2010)

June 16-19
Beijing, China
World Congress of Cardiology Scientific Sessions

June 17-19
Port El Kantaoui, Tunisia
The 7th Tunisian and Europeans Days of Cardiology Practice

July 1-3
Singapore, Singapore
6th Asian Interventional Cardiovascular Therapeutics Congress

July 16-19
Berlin, Germany
Frontiers in CardioVascular Biology 2010-1st Meeting of the CBCS of the ESC

July 24-27
Vancouver, Canada
15th World Congress on Heart Disease, Annual Scientific Sessions 2010

August 13-15
Krabi, Thailand
East Meets West Cardiology 2010

September 16-18
Athens, Greece
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September 30-October 2
Berlin, Germany
5th International Symposium on Integrated Biomarkers in Cardiovascular Diseases

October 10-13
Rochester, United States
26th Annual Echocardiography in Pediatric and Adult Congenital Heart Disease Symposium

October 16-19
Copenhagen, Denmark
Acute Cardiac Care 2010

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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