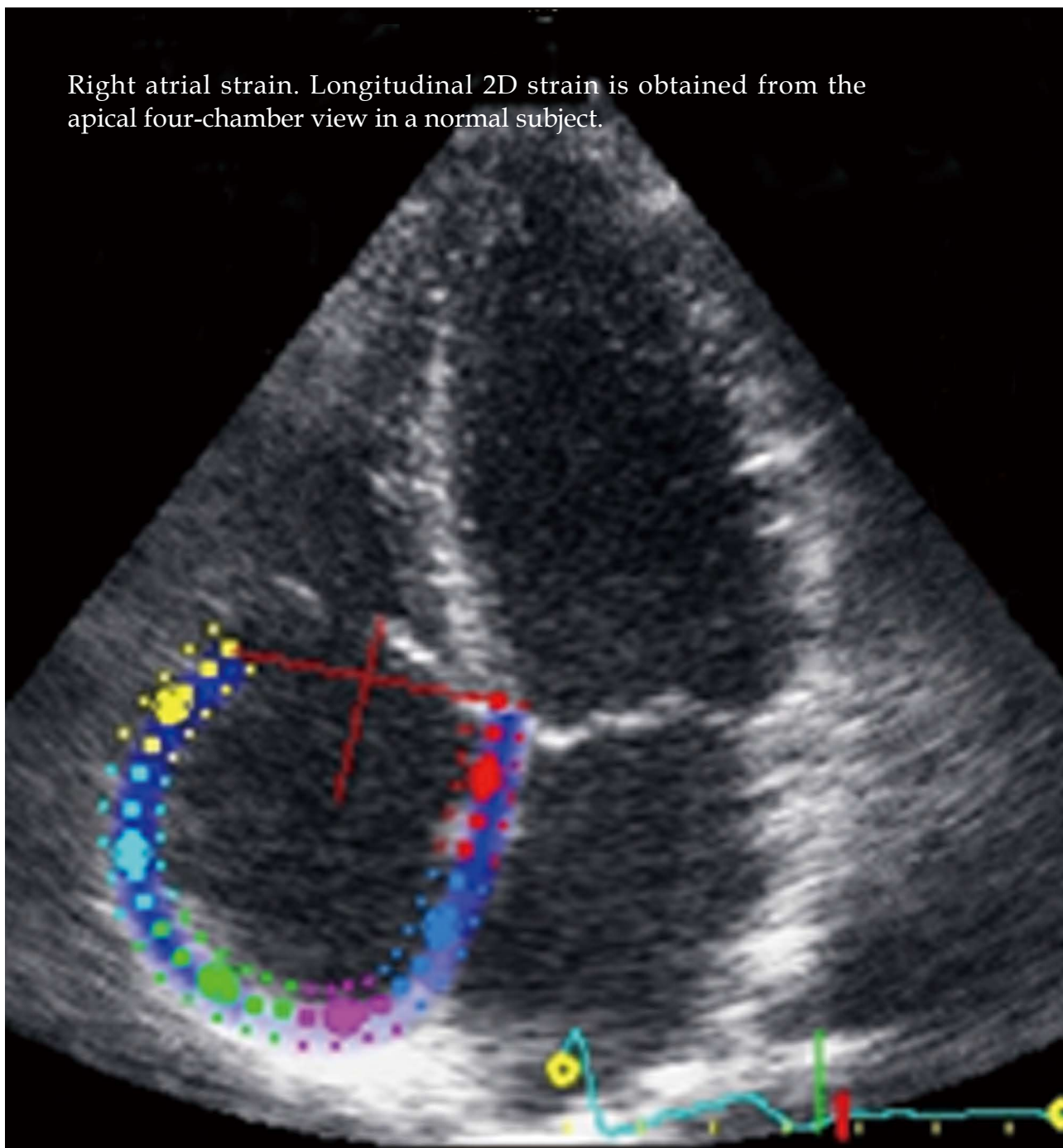




Right atrial strain. Longitudinal 2D strain is obtained from the apical four-chamber view in a normal subject.





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Alternatives to clopidogrel for acute coronary syndromes: Prasugrel or ticagrelor?

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Abstract

Clopidogrel is a mainstay in the treatment of patients with acute coronary syndromes or those receiving endovascular prostheses. However, its efficacy has been challenged in the recent past by studies suggesting variable individual responsiveness and by new, more potent competitors, such as prasugrel and ticagrelor. But what is the actual body of evidence in support of clopidogrel? Is there any dark side of the moon? What is the role of prasugrel, which has already been approved in Europe and in the United States? And what will be the future role of ticagrelor, when approved for routine clinical practice? We hereby concisely summarize the scope of this clinical choice, providing arguments in favor and against each of the three antiplatelet agents: clopidogrel, prasugrel, and ticagrelor.

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Key words: Acute coronary syndrome; Clopidogrel; Prasugrel; Ticagrelor

Peer reviewers: Shinji Satoh, MD, PhD, Department of Cardiology and Clinical Research Institute, National Hospital Or-

THE WELL-KNOWN PAST: CLOPIDOGREL

The crucial role of clopidogrel in association with aspirin in patients with acute coronary syndromes (ACS) is testified by the fact that this drug is among the best selling drugs worldwide, together with statins, proton-pump inhibitors, and angiotensin-II receptor antagonists.

Clopidogrel is a thienopyridine which selectively and irreversibly inhibits the platelet adenosine 5'-diphosphate (ADP) P2Y₁₂ receptor, providing synergistic inhibitory effects on platelet aggregation. Several studies strongly support the favorable risk-benefit balance of clopidogrel in ACS patients managed conservatively as well as invasively, and the most important of these is the Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) trial, which randomly assigned 12 562 patients with ACS to receive clopidogrel (300 mg loading followed by 75 mg once daily) or placebo in addition to aspirin for 3 to 12 mo^[1]. As the devil is often in the details, it is timely to review the main findings of this study. After an average follow-up of 9 mo, the composite event of death from cardiovascular causes, myocardial infarction or stroke occurred in 9.3% vs 11.4%, respectively ($P < 0.001$), a difference largely driven by significantly fewer myocardial infarctions in those treated with clopidogrel (5.2% vs 6.7%, $P < 0.001$). Conversely, the number of deaths from

Table 1 Alternatives to clopidogrel for patients with acute coronary syndromes

Drug	Main features	Pros	Cons
Cilostazol	Phosphodiesterase inhibitor with antiplatelet and antirestenotic effects, also indicated for the medical treatment of claudication	Different mechanism of action translates into increased antithrombotic efficacy when used in combination with aspirin and a thienopyridine	Tolerability limited by gastro-intestinal side effects in up to 20% of patients
Oral anticoagulants	Several agents directly or indirectly inhibiting the coagulation process, including warfarin and dabigatran	Different mechanism of action translates into increased antithrombotic efficacy when used in combination with aspirin	Specificity for the coagulation process translates into lower efficacy on thrombotic processes largely dependent on platelets (such as stent thrombosis). Narrow therapeutic window and need for frequent monitoring (warfarin) translates in higher incidence of bleeding complications
Prasugrel	Third-generation thienopyridine irreversibly inhibiting the P2Y ₁₂ receptor, with quicker, more consistent and more potent action than clopidogrel	Potency and consistency of effect enable homogeneous and nearly complete platelet aggregation inhibition in most patients, with ensuing benefits on myocardial infarction and stent thrombosis	Greater potency may translate into bleeding risk overcoming ischemic benefits in those at moderate or high bleeding risk, such as the elderly and those with previous stroke or transient ischemic attack
Ticagrelor	Non-thienopyridine agent reversibly inhibiting the P2Y ₁₂ receptor, with quicker, more consistent, and more potent action, but shorter half-life than clopidogrel	Direct action translates into quicker onset of action and lack of interaction with drugs metabolized by cytochrome P450, such as proton pump inhibitors	Shorter half-life may translate into greater risk of thrombotic recurrences in case of non-compliance
Ticlopidine	First-generation thienopyridine irreversibly inhibiting the P2Y ₁₂ receptor, with longer half-life than clopidogrel	Limited cross-unresponsiveness translates into potential role in those lacking complete response to clopidogrel. Off-patent status translates into low cost	Lower tolerability with frequent gastro-intestinal adverse effects. Rarely but significantly associated with life-threatening agranulocytosis and thrombotic thrombocytopenic purpura

cardiovascular causes or stroke, when analyzed individually, was not significantly different in the clopidogrel *vs* placebo group (5.1% *vs* 5.5%, $P = 0.3$ and 1.2% *vs* 1.4%, $P = 0.4$, respectively), a key negative finding for the interpretation of more recent trials. In addition, there were significantly more protocol-defined major bleedings in the clopidogrel group (3.7% *vs* 2.7%, $P = 0.001$), despite similar rates of major bleeding defined according to the Thrombolysis in Myocardial Infarction (TIMI) trial (1.1% *vs* 1.2%, $P = 0.7$) or major bleeding related to coronary artery bypass grafting (CABG, 1.3% *vs* 1.1%, $P = 0.3$).

Clopidogrel is also beneficial in patients with acute ST-elevation myocardial infarction (STEMI) managed with thrombolysis, as reported by the Clopidogrel as Adjunctive Reperfusion Therapy-TIMI 28 study and the Clopidogrel and Metoprolol in Myocardial Infarction Trial (COMMIT)^[2,3]. Finally, interventional cardiologists and all endovascular specialists exploit daily the antiplatelet efficacy of clopidogrel in preparation and after deployment of metallic endovascular prostheses, such during percutaneous coronary intervention^[4], transcatheter aortic valve replacement^[5], percutaneous closure of patent foramen ovale^[6], and so forth. Conversely, ACS stemming from coronary vasospasm or local allergic reactions (Kounis syndrome) are unlikely to benefit from antithrombotic therapy, including clopidogrel, unless thrombus is superimposed^[7,8].

Despite such prominent role in the management of subjects with atherothrombosis, including patients with only mildly significant coronary atherosclerosis^[1], and

those treated with implantable cardiovascular devices, clopidogrel has recently been challenged by more potent and, in selected cases, equally safe, antithrombotic agents (Table 1). Besides oral anticoagulants, such as warfarin and the more recent dabigatran^[9], and niche agents, such as cilostazol and ticlopidine^[10,11], the most promising alternatives to clopidogrel in those with background aspirin therapy are prasugrel and ticagrelor.

THE PRESENT: PRASUGREL

Prasugrel is a thienopyridine ADP receptor inhibitor, which irreversibly binds to the P2Y₁₂ receptor. In comparison to clopidogrel, prasugrel acts more quickly, more consistently, more potently, and has been shown to best in pharmacokinetics studies clopidogrel, even when the latter is administered in high loading doses such as 600 or 900 mg. The pivotal trial appraising the role of prasugrel in patients with ACS, including STEMI, is the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-TRITON-TIMI 38^[12]. In this randomized study, including 13 608 patients treated with a 60 mg loading dose and a 10 mg daily maintenance dose of prasugrel or a 300 mg loading dose and a 75 mg daily maintenance dose of clopidogrel for 6 to 15 mo, prasugrel proved more effective than clopidogrel in reducing the risk of death from cardiovascular causes, myocardial infarction, or stroke (9.9% *vs* 12.1%, $P < 0.001$), an effect mainly driven by reduction in myocardial infarctions (7.3% *vs* 9.5%, $P < 0.001$),

actually often qualifying for stent thrombosis (1.1% *vs* 2.4%, $P < 0.001$). Indeed, the authors reported similar rates of death from any cause (3.0% *vs* 3.2%, $P = 0.6$), death from cardiovascular causes (2.1% *vs* 2.4%, $P = 0.3$), and stroke (1.0% *vs* 1.0%, $P = 0.9$) in the prasugrel *vs* clopidogrel groups. This remarkable antithrombotic effects were however offset by an increased bleeding risk, clustering particularly in the elderly and those with previous stroke or transient ischemic attack, as major bleeding occurred in 2.5% of those treated with prasugrel *vs* 1.7% of those treated with clopidogrel ($P = 0.001$), with the excess risk mainly due to CABG-related major bleeding (0.4% *vs* 0.1%, $P = 0.001$).

THE FUTURE: TICAGRELOR

Ticagrelor is instead a reversible inhibitor of platelet P2Y₁₂-subtype ADP receptor, and thus does not belong to the thienopyridine family. Given its reversible binding to the target receptor and shorter half-life, ticagrelor holds the promise of a larger therapeutic window, especially for patients who might end up undergoing CABG early after drug administration. Indeed, the pivotal Platelet Inhibition and Patient Outcomes (PLATO) study randomized 18624 patients with ACS to 180 mg loading dose, 90 mg twice daily thereafter of ticagrelor *vs* 300-600 mg loading dose, 75 mg daily thereafter of clopidogrel for 12 mo^[13]. The risk of death from vascular causes, myocardial infarction, or stroke was significantly reduced by ticagrelor (9.8% *vs* 11.7%, $P < 0.001$), an effect stemming from consistent reductions in the risk of death from all causes (4.5% *vs* 5.9%, $P < 0.001$), death from vascular causes (4.0% *vs* 5.1%, $P = 0.001$), and myocardial infarction (5.8% *vs* 6.9%, $P = 0.005$), including stent thrombosis (1.3% *vs* 1.9%, $P = 0.009$). Stroke occurred with similar frequency in the ticagrelor and clopidogrel groups (1.5% *vs* 1.3%, $P = 0.2$), similarly to CABG-related major bleeding (4.8% *vs* 5.2%, $P = 0.3$) and all TIMI major bleeding (7.1% *vs* 6.9%, $P = 0.7$). However, non-CABG related bleeding still occurred more frequently in the ticagrelor group (2.8% *vs* 2.2%, $P = 0.030$).

RECONCILING THE EVIDENCE

Awaiting head-to-head randomized trials of ticagrelor *vs* prasugrel, it is difficult to identify which of these two agents offers the best risk-benefit balance to overcome the limitations of clopidogrel. A superficial review of the PLATO and TRITON-TIMI 38 trials would suggest that ticagrelor is the winner in most patients, including those at moderately increased bleeding risk, given the significant mortality benefit and the similar risk of CABG-related major bleeding. However, formal interaction tests would probably provide more precise adjusted indirect comparison estimates, enabling decision makers to select the most appropriate agent for each individual clinical case, in order to maximize safety but also efficacy^[14,15]. Indeed, the dramatic reduction in the

risk of stent thrombosis, especially drug-eluting stent thrombosis, achieved by prasugrel (0.8% *vs* 2.3%, $P < 0.001$), would suggest that this agent should probably be considered the first-line one in those at higher risk of thrombotic events^[16], such as diabetics and/or those with diffuse coronary stenting^[17].

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Aortic stenosis: An update

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Abstract

Aortic stenosis (AS) is the most common valvular heart disease in the world. It is a disease of the elderly and as our population is getting older in both the developed and the developing world, there has been an increase in the prevalence of AS. It is impacting the mortality and morbidity of our elderly population. It is also causing a huge burden on the healthcare system. There has been tremendous progress in our understanding of AS in recent years. Lately, studies have shown that AS is not just a disease of the aortic valve but it affects the entire systemic vasculature. There are studies looking at more sophisticated measures of disease severity that might better predict the optimal timing of valve replacement. The improvement in our understanding in etiology and pathophysiology of the disease process has led to a number of trials with possible treatment options for AS. In this review, we talk about our understanding of the disease and latest developments in disease assessment and management. We look forward to a time when there will be medical treatment for AS.

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INTRODUCTION

Aortic stenosis (AS) is the most common valvular heart disease and the third most common cardiovascular disease after hypertension and coronary artery disease in the western world^[1]. The prevalence of AS increases from 2% in adults over 65 years to 4% in adults over 85 years of age^[2]. AS is a progressive condition and after the onset of heart failure, survival is < 2 years without valve replacement^[3]. 50% of patients with AS presenting with angina, syncope or heart failure survive for 5, 3 or 2 years respectively without aortic valve replacement (AVR). As life spans increase, the burden of senile AS on the health care system is expected to increase. Close monitoring and use of AVR when the disease becomes significant remains the standard of care^[4,5]. AS is the most common reason for AVR in the developed world. Though the disease has been known for decades, little has been known about the pathophysiology, and a lot of work is going into the understanding of possible etio-pathogenesis. New innovative techniques and management have been studied to stop or reduce the progression of AS. To date though, no medical therapy has been proven to alter the natural history of patients with AS. In this review, we talk about our understanding of the disease and latest developments in its management.

ETIOLOGY OF CALCIFIC AS

AS was always thought to be due to a passive, degenerative process leading to accumulation of calcium and causing narrowing of the valve. The pathogenesis of AS, however, is still not well understood. The risk factors for calcific aortic valve disease are similar to the risk factors for atherosclerosis, which includes male sex, hypertension, elevated levels of low density lipoprotein (LDL) cholesterol, and smoking^[2,6-8]. The incidence of AS is higher in patients with chronic kidney disease and patients who have had radiotherapy in the past. It has been shown from the valve specimens taken during surgery for AS that there are increased levels of inflammation, which might lead to calcification^[9-12]. There are also increased levels of LDL, which might lead to inflammation and calcification^[13,14].

Recent studies have shown specific bone-cell phenotypes are present in calcifying human valves^[15,16]. Specific markers of bone formation have been identified in calcified aortic valves; it includes the bone matrix proteins osteopontin, osteocalcin and bone sialoprotein^[17-22], and the osteoblast transcription factor Runx2^[18,23,24]. Genetic studies are also evolving, which point towards genetic factors that predispose individuals to developing calcific AS.

DIAGNOSIS

Patients may present with symptoms of exertional chest pain, breathlessness or syncope but the majority of patients are asymptomatic. AS is suspected when an ejection systolic murmur (ESM) is heard in the precordium in symptomatic or asymptomatic patients. Diagnosis of AS is by detailed history and physical examination, echocardiography and confirmation with cardiac catheterization.

Clinical evaluation

History and physical examination is very important in patients with AS. It helps not only for the diagnosis but also to assess severity. Patients with AS usually have ESM in the precordium. Patients with exertional symptoms have hemodynamically significant valvular stenosis and hence need treatment. The severity of AS in asymptomatic patients can be assessed by other clinical findings - poorly palpable pulse, late peaking of ESM and reversal of S2 split.

Echocardiography

Echocardiography is the most commonly used noninvasive test of choice for patients with suspected AS. The severity of AS is determined by echocardiography by the mean and peak aortic valve gradients (AVG), aortic valve area (AVA) and aortic valve velocity. Echocardiography does not measure the pressures directly and the AVA is also a derived value^[25]. The AVG is also dependent on stroke volume and systolic ejection time. Hence the hemodynamic measurements and calculations change rapidly. There is a possibility of underestimation of AVA if

the ejection fraction is reduced. AS is a systemic vascular disease affecting not only the valve but also the systemic vasculature. More sophisticated measures of disease severity are needed to explain the overlap in hemodynamic severity between symptomatic and asymptomatic patients. In this way we might be able to better predict the optimal timing of AVR. New mathematical models are being studied to look at ventricular-vascular coupling in the accurate assessment of severity of AS^[26]. The other role of echocardiography is the use of stress echocardiography in moderate stenosis to see if the patient might benefit from AVR.

Cardiac catheterization

Cardiac catheterization is essential in almost all patients with AS. It is useful in confirming the severity of disease and it is also useful if there is discordance between clinical examination and Doppler measurements. Cardiac catheterization allows for actual measurement of the AVG and cardiac output, and calculation of the AVA. We can also measure the atrial and ventricular volumes, cardiac output and right heart pressures. Cardiac catheterization with selective coronary arteriography is necessary to diagnose the presence, location and severity of associated coronary artery disease.

Multidetector computed tomographic and cine magnetic resonance

Multidetector computed tomography (CT) has become far more than a simple anatomic technique dedicated to coronary imaging. Cine magnetic resonance (MR) use in the diagnosis of cardiac diseases has been expanding. Both multidetector CT and cine MR have been used in the assessment of contractile function and for characterization of myocardial infarction. They may also provide important information pertaining to valve morphology, accurately measuring the AVA and in the assessment of severity of stenosis^[27].

MANAGEMENT

The AVA normally ranges from 3.0 to 4.0 cm² in adults and a transvalvular gradient usually develops when the orifice area is < 50% of normal. In patients with normal left ventricular systolic function, severe AS is defined as a peak AS velocity > 4 m/s, a mean transaortic pressure gradient > 40 mmHg, or an AVA < 1 cm². A valve area index < 0.6 cm²/m² is also indicative of severe AS^[4,5]. The management of severe AS has historically been surgery, but other treatment options have been looked at.

Hydroxymethylglutaryl-coenzyme A reductase inhibitors

Lipids are known to be the key in the development of fibrosis and then calcification of aortic valves leading to stenosis. Hence lipid lowering agents, hydroxymethylglutaryl-coenzyme A reductase inhibitors, or statins, may be a potential agent for halting the progression of AS. Studies have shown variable results.

The Rosuvastatin Affecting Aortic Valve Endothelium study was a prospective study looking at 121 patients with asymptomatic moderate to severe AS with AVA of 1.0 to 1.5 cm² and followed them by echocardiogram. Patients with a LDL cholesterol > 3.4 mmol/L were treated with rosuvastatin while those with an LDL < 3.4 mmol/L received no lipid lowering therapy. Over a mean follow-up of 73 wk there was reduced progression of AS in the rosuvastatin group compared to the control group (increase in AS velocity of 0.04 m/s per year in the rosuvastatin group *vs* 0.24 m/s per year in the control group, $P = 0.007$; decrease in AVA of 0.05 cm² per year in the rosuvastatin group *vs* 0.10 cm² per year in the control group, $P = 0.041$). This study showed promise for statins in reducing progression of AS^[28].

The Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression study was a randomized trial on 155 patients who received either atorvastatin 80 mg daily or placebo. Patients with increased peak AS velocity and aortic valve calcification on echocardiography were enrolled. The average peak AS velocity was 3.43 m/s, the average AVA was 1.03 cm², and aortic valve calcium score was 5920 log arbitrary units. Thirty-six patients had severe AS based on a peak AS velocity. Patients were followed for a median of 25 mo. Despite a significant change in the mean LDL cholesterol between the two groups following treatment ($P < 0.001$), there was no difference in measures of AS progression between the two groups (increase in peak aortic jet velocity of 0.20 m/s in both groups, $P = 0.95$; increase in valvular calcification 22.3% per year in the atorvastatin group *vs* 21.7% per year in the placebo group, $P = 0.93$)^[29].

Simvastatin and Ezetimibe in Aortic Stenosis was a randomized, double-blind trial involving 1873 patients with mild-to-moderate asymptomatic AS. The patients received either 40 mg of simvastatin plus 10 mg of ezetimibe or placebo daily. The primary outcome was a composite of major cardiovascular events, including death from cardiovascular causes, AVR, nonfatal myocardial infarction, and hospitalization for unstable angina pectoris, heart failure, coronary artery bypass grafting, percutaneous coronary intervention, and nonhemorrhagic stroke. During a median follow-up of 52.2 mo, the primary outcome occurred in 333 patients (35.3%) in the simvastatin-ezetimibe group and in 355 patients (38.2%) in the placebo group [hazard ratio in the simvastatin-ezetimibe group, 0.96; 95% confidence interval (CI): 0.83-1.12, $P = 0.59$]. AVR was performed in 267 patients (28.3%) in the simvastatin-ezetimibe group and in 278 patients (29.9%) in the placebo group (hazard ratio, 1.00; 95% CI: 0.84-1.18, $P = 0.97$)^[30]. This study showed that cholesterol lowering medications, simvastatin and ezetimibe did not reduce the composite outcome of combined aortic valve events. This therapy reduced the incidence of ischemic cardiovascular events but not events related to aortic valve stenosis.

The Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin trial is a randomized,

double-blind, placebo-controlled trial in asymptomatic patients with mild to moderate AS and no clinical indications for cholesterol lowering. The patients were randomized to receive either placebo or rosuvastatin. A total of 269 patients were randomized: 134 patients to rosuvastatin 40 mg daily and 135 patients to placebo. The median follow-up was 3.5 years. The annualized increase in the peak AS gradient was 6.3 mmHg in the rosuvastatin group and 6.1 mmHg in the placebo group ($P = 0.83$)^[31]. Hence this was again a negative study for use of statins in AS.

The effect of statins on the progression of AS is still not clear. The latest trials do not show any benefit in established AS. We may have to look at high risk patients like possible gene linkage or patients with end-stage renal disease and trying to prevent AS. We might also have to look at patients with mild disease and see if it reduces the disease progression. Further studies are required to answer our questions.

Angiotensin-converting enzyme inhibitors

Angiotensin-converting enzyme (ACE) and angiotensin II receptors have been found in stenotic aortic valves. This leads to the hypothesis that the renin-angiotensin system may play a role in disease progression. This led to trials with ACE inhibitors (ACEi) looking for their effect in AS. Two hundred and eleven patients with asymptomatic AS and a peak AS velocity of > 2.5 m/s (average peak AS velocity 3.96 m/s; mean AVA 0.84 cm²) were retrospectively identified and the rates of hemodynamic progression of AS were compared between patients who were taking an ACEi *vs* those who were not^[32]. No difference was found (increase in peak AS velocity of 0.29 m/s per year *vs* 0.35 m/s per year, respectively, $P = 0.29$). To date, there is no randomized trial looking at the effect of ACEi in AS.

Bisphosphonates

Rajamannan *et al*^[15] showed that the mechanism for aortic valve calcification is similar to that for skeletal bone formation and that this process is mediated by an osteoblast-like phenotype. Innasimuthu *et al*^[33,34] did a retrospective study on patients > 70 years, who had transthoracic echocardiograms (TTE) > 1 year apart and an initial AVA of 0.6-2.0 cm². Patients were excluded if they had an ejection fraction < 40%, other significant valvular or congenital heart disease, end-stage renal disease or heart transplant. The cohort was divided depending on the use of bisphosphonates. AVA, peak and mean aortic valve gradient, and the change between the studies were calculated. Seventy six patients fit study criteria with 8 in the bisphosphonate group and 68 in the non-bisphosphonate group. The period between the TTEs was 23 ± 5 mo in both the groups. AVA in the non-bisphosphonate group worsened by 0.2 cm² and in the bisphosphonate group it improved by 0.1 cm² ($P = 0.001$)^[33,34].

Skolnick *et al*^[35] did an observational study of patients with AS from the echocardiographic database comparing

18 patients on treatment for osteoporosis (bisphosphonates, calcitonin, or estrogen receptor modulators) with 37 patients not on the treatment. AVA was calculated using the continuity equation. Mean baseline AVA was 1.33 cm² and not significantly different between groups. After a mean of 2.4 years, mean annual changes in AVA were reduction by 0.22 cm² in those not on treatment for osteoporosis and 0.10 cm² in patients receiving osteoporosis treatment ($P = 0.025$). In a multivariable analysis including age, gender, and statin use, only the treatment group was associated with a change in AVA^[35]. There has recently been increased interest in bisphosphonates as a medication in reducing the disease progression. These two studies are retrospective and used observational data and hence randomized trials are required to assess its effect on AS.

Transcatheter aortic valve implantation

In 2002, the first patient underwent transcatheter aortic valve implantation (AVI) for the treatment of severe symptomatic AS. There have been studies looking at the feasibility of percutaneous transvenous, transarterial, and transapical placement of the aortic valve. There were several single-center trials, which demonstrated that this new approach was a reasonable treatment option for patients who were inoperable or at a very high risk for surgery. The results of recent multicenter trials have shown that the procedure is safe and effective. These were associated with success rates of > 90% and 30-d procedural mortality rates of < 10% even though the trials involved very high-risk patients^[36,37]. The prospective randomized PARTNER study, whose results will be available towards the end of 2010, will make a significant contribution to clearly establishing the safety and efficacy of percutaneous placement of the aortic valve in patients who are inoperable or at a high surgical risk.

Surgery

AVR is the definitive therapy for severe AS. Over time, the operative risk has dramatically decreased; currently operative mortality of isolated AVR is 2%-5% in patients over 70 years and 5%-15% in older adults. After valve replacement, symptoms diminish, quality of life improves, and long-term survival is similar to that expected for an age-matched population^[38,39]. Patient-prosthesis mismatch could result in significant mortality and morbidity in patients after AVR. Hence careful selection of prostheses is important for the longevity of the replaced valve^[40,41].

CONCLUSION

AS is a very common valvular heart disease and it can lead to significant mortality and morbidity in the elderly population. Severity of the disease is estimated by symptoms, clinical evaluation, echocardiography and cardiac catheterization. We need more studies looking at new techniques to assess the severity of AS more accurately

and to predict the optimal timing of surgery. The only known effective treatment with documented benefits is AVR. There are a number of studies looking at the possible benefit of medications in reducing the progression of AS and transcatheter AVI in inoperable patients. We need more randomized controlled trials looking at the effective use of medications and minimally invasive procedures.

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Coronary heart disease: Significance of liver X receptor α genomics

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Abstract

Crosstalk between lipid peroxidation and inflammation is known to be a pathognomonic feature for the development of coronary heart disease (CHD). In this regard ligand activated liver X receptor (LXR)- α has emerged as a key molecular switch by its inherent ability to modulate an array of genes involved in these two fundamental cellular processes. In addition, LXR- α has also been found to play a role in hepatic lipogenesis and innate immunity. Although several lines of evidence in experimental model systems have established the atheroprotective nature of LXR- α , human subjects have been reported to possess a paradoxical situation in which increased blood cellular LXR- α gene expression is always accompanied by increased coronary occlusion. This apparent paradox was resolved recently by the finding that CHD patients possess a deregulated LXR- α transcriptome due to impaired ligand-receptor interaction. This blood cellular mutated LXR- α gene expression correlated specifically with the extent of coronary occlusion and hence need is felt to devise new synthetic ligands that could restore the function of this mutated LXR- α protein in order to modulate genes involved in reverse cholesterol transport and suppression of the inflammatory response leading to the effective treatment of CHD.

INTRODUCTION

In the last decade an accumulation of data from *in vitro* to *in vivo* model systems has established a pivotal role of liver X receptor (LXR) gene for its ability to regulate two fundamental cellular processes i.e. lipid metabolism and inflammation, which are the key components for the pathogenesis of coronary heart disease (CHD). There is a general recognition of the fact that co-operativity between lipid peroxidation and inflammation initiates a complex interaction between blood mononuclear cells, blood derived factors and the arterial wall leading to the development of CHD^[1-3]. At the molecular level such a phenomenon arises due to interplay of genes involved in lipid metabolism and inflammation. In the light of this cross-talk, the LXR- α molecule has caught the imagination of researchers across the globe due to its inherent capacity to modulate an array of genes involved in various cellular metabolic processes^[4]. In this review, an attempt will be made to evolve the interrelationship of LXR- α genomics with lipid peroxidation, inflammation and human atherosclerosis, a phenomenon vital for the design of an LXR- α gene based preventive/therapeutic strategy against CHD.

ATHEROGENESIS: LIPID PEROXIDATION MEETS INFLAMMATION

Much of the recent research on the origin of atherosclerosis has concentrated on the interplay between lipid metabolism, cytokines and cellular activity within arterial wall. Oxidative modification of low density lipoprotein (LDL) and 'response to injury' are two fundamental mechanisms proposed to be involved in the pathogenesis of atherosclerosis. Oxidized LDL has been shown to bypass the negative feedback mechanism in macrophages that inhibits the excessive uptake of native LDL^[5]. Additionally, a vast number of oxidation products of lipids and proteins have been demonstrated in atherosclerotic lesions and in the plasma of patients with atherosclerosis^[6-8]. On the other hand according to the 'response to injury hypothesis'^[9], endothelial nudation is the initial step in atherosclerosis, however recent research has focused on endothelial dysfunction rather than endothelial nudation as being the first pathognomonic feature of atherosclerosis^[10]. The risk factors influencing the development of atherosclerosis mainly involve hypercholesterolemia, altered LDL, hypertension, elevated levels of homocystine, genetic alteration, smoking, diabetes mellitus and infectious microorganisms such as *Chlamydia pneumoniae*^[11].

The first step in the pathogenesis of initial lesion development is lipid deposition followed by monocyte recruitment to the sub-endothelial space which is regulated by nuclear factor κ B (NF- κ B) induced gene expression^[1]. At first LDL shuttling in and out of the vessel wall are trapped as a result of oxidative modifications by myeloperoxidase and 12/15 lipoxygenase enzymes^[12,13]. Trapped LDL aggregates undergo further oxidation and oxidized LDL induces expression of adhesion factors in an NF- κ B dependent manner^[14]. Further monocyte attach to vascular endothelial cells which is mediated by selectins^[15], vascular cell adhesion molecule-1 and intracellular cell adhesion molecule-1^[16]. Monocyte transmigration into the sub-endothelial space is mediated by endothelium monocyte chemoattractant protein-1 (MCP-1) and its receptor CCR-2^[17,18]. In the subendothelial space monocytes differentiate into macrophages under the influence of monocyte colony stimulating factor and express CD36, scavenger receptor-A which specifically recognizes oxidized LDL. Uptake of oxidized LDL by macrophages leads to foam cell formation, which is a crucial landmark for the pathogenesis of atherosclerosis. Lipid loaded macrophages in the subendothelial space release reactive oxygen species and cytokines which leads to further expression of adhesion factors and subsequent monocyte migration and exacerbation of vascular inflammation. Macrophages may also be an active player in both intracellular and extracellular lipid peroxidation. Foam cells also mediate the release of reactive oxygen species and inflammatory cytokines and oxidation of lipoprotein particles in the subendothelial space^[19-23]. Thus co-operativity between lipid peroxidation and inflamma-

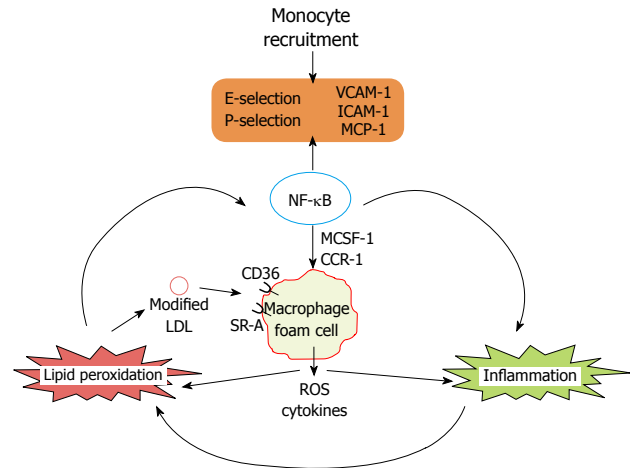


Figure 1 Lipid peroxidation vs inflammation in the development of coronary heart disease. VCAM-1: Vascular cell adhesion molecule-1; ICAM-1: Intracellular cell adhesion molecule-1; MCP-1: Monocyte chemoattractant protein-1; MCSF-1: Monocyte colony stimulating factor; LDL: Low density lipoprotein; NF- κ B: Nuclear factor κ B; ROS: Reactive oxygen species.

tion is a hallmark for the development of atherosclerosis (Figure 1).

In summary, endothelial dysfunction followed by co-operative action of lipid peroxidation and inflammation on vascular wall leads to the initial lesion development, mediated by the NF- κ B dependent expression of various cell adhesion molecules and mediators which leads to the recruitment of monocytes and furthers their differentiation into macrophage foam cells is the hallmark feature for the development of CHD.

LXR- α EPIGENOMICS FROM BASIC BIOLOGY TO CLINIC

Based upon the sequence homology with other nuclear receptors LXRs (LXR- α , NR1H3 and LXR- β , NR1H2) were cloned a decade ago and considered originally as "orphan" nuclear receptors as their natural ligands were unknown^[24,25]. LXR- α is highly expressed in liver, adipose tissue and macrophages whereas the β isoform is ubiquitously expressed in all tissues^[26]. The LXRs are ligand activated transcription factors that form permissive heterodimers with retinoid X receptor (RXR). This heterodimer binds to LXR response element in the DNA consisting of direct repeats of the core sequence AG-GTCA separated by four nucleotides^[27]. In the absence of ligand, LXRs recruit complexes of co-repressors that are substituted by co-activators upon ligand binding^[28] and thus regulate the gene expression (Figure 2).

Mono-oxygenated derivatives of cholesterol or oxysterols are the major physiological LXR ligands. Intermediates between steroid hormone synthesis, 22(R) hydroxy cholesterol and 20(S) hydroxy cholesterol, have been shown to bind and stimulate LXR- α transcriptional activity in the physiological range. In the brain 24(S) hydroxy cholesterol and in the liver 24(S),25-epoxycholesterol is

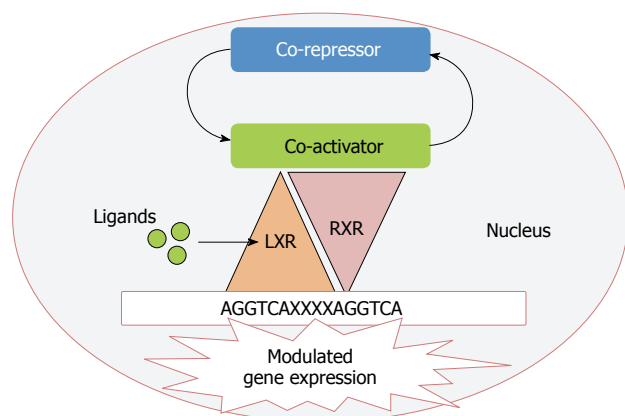


Figure 2 Mechanism for the transcriptional regulation by liver X receptor (LXR). RXR: Retinoid X receptor.

the abundant LXR agonist^[29-32]. Apart from these, studies have demonstrated that D-glucose and D-glucose 6 phosphate are endogenous LXR agonists with equal efficacy to that of oxysterols^[33], however recent findings have questioned this fact on the basis of inability of glucose and its metabolites to influence the interaction of cofactors with LXR and the lack of involvement of LXRs in the regulation of glucose sensitive genes in the liver^[34]. Based upon molecular docking and *in vitro* studies, our recent finding have revealed withaferin A as a novel LXR- α agonist, which interacts in a similar fashion within the ligand binding domain, as its natural physiological ligands and these have potential to activate LXR- α ^[35]. An indol alkaloid, paxilline, produced by a fungus *Penicillium paxilli* was the first natural non oxysterol LXR agonist^[36] but due to its toxicity in *in vivo* studies it is unsuitable. Riccardin C is also a natural nonsteroidal compound isolated from liverworts, which acts as an LXR- α agonist and LXR- β antagonist^[37]. Synthetic LXR agonists T0901317 and GW3965 are commonly used in experimental studies. In addition to LXR agonists geranyl-geranyl pyrophosphate has been found to inhibit LXR activity by antagonizing their interaction with coactivators^[38,39]. Further transcriptional activity of LXR has also been shown to be inhibited by oxidized cholesterol 3 sulphates (normally found in human plasma) and polyunsaturated fatty acids in various cell lines^[40,41] (Figure 3).

Studies on LXR- α ^{-/-} mice, but not LXR- β ^{-/-} mice, showed a marked cholesterol ester accumulation in the liver when fed with diets containing cholesterol^[42]. This led to the identification of the first known LXR- α direct target CYP7A1 (the rate limiting enzyme in bile acid synthesis). The different phenotypes of the two knockout mice strains indicates that despite the considerable sequence homology between two LXR isoforms they have different distinct biological functions. With the identification of several LXR- α target genes, this molecule became a fascinating player for understanding its role in the mechanism of regulation of macrophage cholesterol metabolism, hepatic lipogenesis, and enterohepatic circulation by inhibiting cholesterol absorption. Apart from

its role in atherosclerosis, recent data have uncovered the role of LXR- α in inflammation and immunity also leading to the integration of three fundamental cellular processes i.e. lipid metabolism, inflammation and immunity (Figure 4).

LXR- α in the trafficking of cholesterol

A primary function of LXR- α is to maintain cellular cholesterol homeostasis by participating in the process of reverse cholesterol transport^[43]. *In vivo* activation of LXR- α with synthetic high affinity ligand increases HDL levels and net cholesterol secretion^[44]. These activities are mediated by LXR- α by upregulating the expression of the ABC superfamily of membrane transporters including ABCA1, ABCG5, ABCG8 and ABCG1^[45-51]. Mutations in the ABCA1 gene are the cause of Tangier disease, characterized by the complete absence of HDL in plasma of afflicted patients, resulting in the accumulation of cholesterol in tissue macrophages and an increased incidence of cardiovascular diseases^[52]. In addition to ABC transporters, LXR- α driven reverse cholesterol is promoted by the induction of a subset of apolipoproteins that serves as cholesterol acceptors. It is now well recognized that in macrophages, which play a central role in the pathogenesis of atherosclerosis^[53], ABCA1 facilitates the efflux of cholesterol and phospholipids to the lipid poor lipoproteins (apoA-I) and its induction may contribute to the increases in the plasma HDL level seen with LXR- α ligand treatment^[54,55]. In addition LXR- α also induces the expression of apoE in macrophages and adipose tissues but not in liver^[56], further LXR- α also induces apoC gene clusters in macrophages and apoD in adipose tissues^[57,58]. The importance of the activation of apoC and apoD by LXR- α in lipoprotein metabolism are unknown but the protective role of apoE in atherogenesis has been uncovered. Loss of macrophage apoE leads to the increased lesion, whereas overexpression of apoE by LXR agonists leads to the reversed phenotype^[59]. Further LXR- α have been shown to modulate the expression of various enzymes that act on lipoproteins including lipoprotein lipase, cholesterol ester transfer protein and phospholipids transfer protein (PLTP)^[60-63]. Thus under increased intracellular cholesterol levels, these pathways would be expected to impact the progression of cardiovascular diseases and LXR- α agonists can be exploited for the therapeutic interventions.

LXRs in hepatic lipogenesis

In addition to their ability to modulate cholesterol metabolism, LXR- α also plays a regulatory role in hepatic lipogenesis. Findings that treatment of mice with LXR agonist elevates triglyceride levels in liver and plasma have raised an obstacle to the development of these compounds as human therapeutics^[64,65]. The primary mechanism by which LXR- α agonists stimulate lipogenesis appears to be through direct activation of the SREBP-1c promoter^[66,67]. Further LXR- α have direct actions on certain lipogenic genes such as fatty acid synthase, PLTP, sterol coenzyme A desaturase 1 and acyl coenzyme A carboxylase^[68].

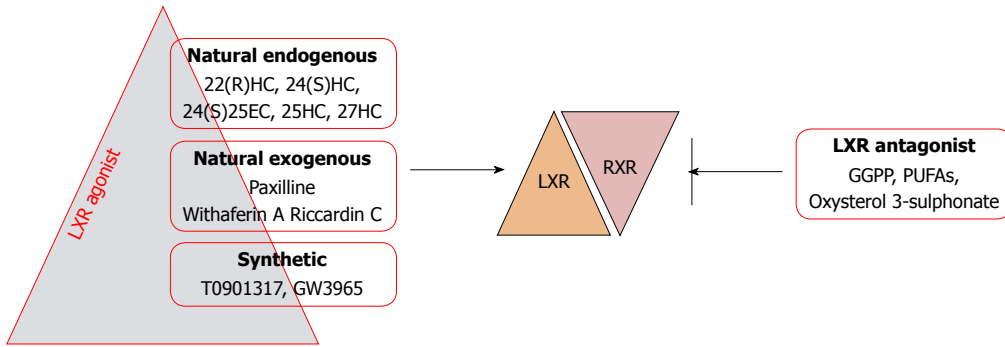


Figure 3 Classification of LXR modulators.

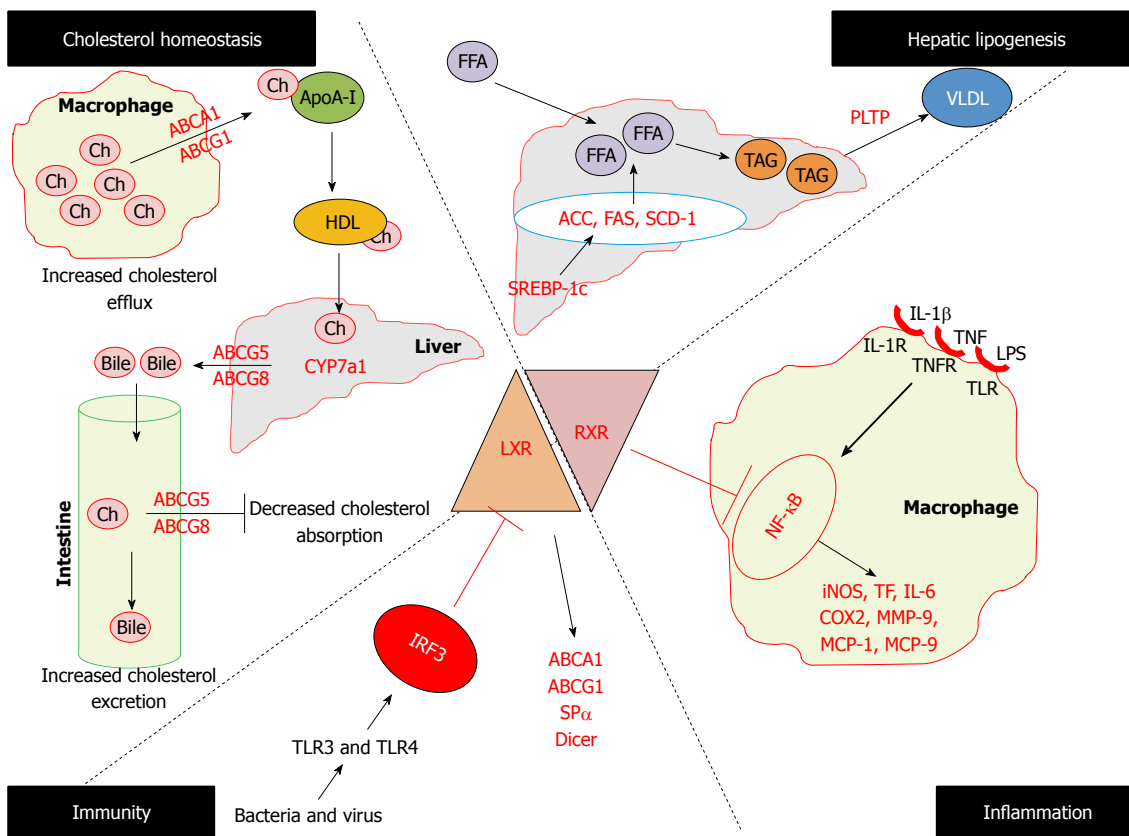


Figure 4 Diverse biological functions of LXRs. ACC: Acyl coenzyme A carboxylase; FAS: Fatty acid synthase; SCD1: Sterol coenzyme A desaturase 1; PLTP: Phospholipids transfer protein; iNOS: Inducible nitric oxide synthase; COX2: Cyclo-oxygenase 2; IL: Interleukin; MMP-9: Matrix mettaloproteinase-9; TNF: Tumor necrosis factor; TF: Tissue factor.

LXR- α in inflammation

Several lines of evidence show that excessive inflammation within the arterial wall is a risk factor for cardiovascular disease and promotes atherogenesis^[53,69]. A growing body of data has indicated that apart from the reverse cholesterol transport, LXR- α reciprocally regulates a set of inflammatory genes after bacterial, LPS, tumor necrosis factor (TNF) or interleukin (IL)-1 β stimulation, such as inducible nitric oxide synthase (iNOS), cyclo-oxygenase 2, IL-6, MCP-1, MCP-9, and matrix mettaloproteinase-9 (MMP-9)^[4,70]. Further in two mouse models of chronic atherogenic inflammation, Apoe^{-/-} and ldlr^{-/-} mice, it has been reported that administration of LXR- α ligands re-

pressed the aortic expression of MMP-9 and tissue factor (TF) while inducing the expression of ABCA1^[4,71]. The mechanisms for the repression of inflammatory genes are not well understood but evidence supports the involvement of the NF- κ B pathway^[72]. Thus all the observations confirm the anti-atherogenic effects of LXR- α agonists not only by promoting cholesterol efflux but also by repressing the inflammatory mediators.

LXR- α in immunity

Recent studies have revealed a common mechanism by which different microbial pathogens might contribute to foam cell formation and accelerated lesion development

by interfering with LXR dependent cholesterol metabolism^[73]. Activation of TLR3 and TLR4 during bacterial or viral infection of macrophages severely compromises the expression of ABCA1, ABCG1 and ApoE and other LXR target genes. TLR3/4 dependent inhibition of LXR is accomplished through activation of viral response transcription factor IFN regulatory response factor-3, however the mechanism by which this factor blocks LXR activation remains to be determined. Further macrophages from Sp α ^{-/-} (antiapoptotic gene) mice are highly susceptible to oxidized LDL loading induced apoptosis *in vitro* and undergo massive apoptosis within atherosclerotic lesions *in vivo*^[74]. A study from our laboratory has revealed for the first time that the LXR- α knock down cellular model has lower expression of the dicer gene, which shows the involvement of LXR- α in RNAi mediated innate immune responses^[75].

LXRs in glucose homeostasis

Identification of LXRs as a mediator of insulin action in the liver, have pointed the role of these receptors in glucose homeostasis. Several studies have demonstrated potent glucose lowering and insulin sensitizing effects of synthetic LXR agonists in various rodent models of diabetes and insulin resistance^[76-78]. It has been demonstrated that LXR activation leads to the suppression of various genes involved in gluconeogenesis (phosphoenolpyruvate carboxykinase, fructose-1,6 biphosphatase, and glucose 6 phosphatase) in the liver of wild type but not in LXR α / β deficient mice^[79]. A further LXR response element was identified in the promoter region of glucose transporter 4 (GLUT4) gene in mice and humans^[78,80] and synthetic LXR agonists were shown to increase GLUT4 expression in white adipose tissues of mice and rats as well as in cultured murine and human adipocytes^[78,80,81-83]. In addition to suppression of gluconeogenesis and increased uptake of peripheral glucose uptake by LXR activators, it was shown that prolonged exposure of rat pancreatic islet insulinoma cell lines to T0901317 increases insulin secretion by glucose and glucagon-like peptide^[84,85]. Thus potent glucose lowering properties of LXR agonists (T0901317 and GW3965) demonstrated in the rodent studies suggest a potential clinical use of LXR agonists as antidiabetic drugs.

In summary, ligand activated nuclear receptor LXR- α maintains cellular cholesterol homeostasis by regulating the genes involved in reverse cholesterol transport as well as hepatic lipogenesis. Further LXR activators have also been found to regulate glucose homeostasis by inhibiting gluconeogenesis and promoting peripheral glucose uptake as well insulin secretion. Apart from the fundamental cellular processes, LXRs reciprocally regulate the genes involved in inflammation.

REGULATION OF LXR- α EXPRESSION AND ACTIVITY

Despite extensive research in the field of LXR biology

very little is known about the regulation of expression and activity of these receptors. LXR- β is constitutively expressed while LXR- α expression can be modulated. There are three LXR- α isoforms. All are derived from the same gene *via* alternative splicing^[86] although relevance of the various isoforms has not yet been characterized. The LXR- α 2 isoform lacks the first 45 amino acids of LXR- α 1 and LXR- α 3 lacks 50 amino acids within the ligand binding domain. LXR- α 2 and LXR- α 3 are expressed at lower levels, except in the testis where LXR- α 2 is predominant. LXR- α 2 shows reduced transcriptional activity and LXR- α 3 is unable to bind ligand and is transcriptionally inactive^[86]. Further in human LXR- α expression can be regulated by the auto regulatory loop mechanism as the LXR- α promoter itself contains its response element^[87]. In addition to responding with their agonist or antagonist or by co-activator and co-repressor, the phosphorylation status of LXR- α also affects its activity. Under basal conditions, LXR- α is phosphorylated at S198 a general target for the mitogen-activated protein kinase (MAPK) family. A phosphorylation site mutant LXR- α remains nuclear and responds to ligands like the wild-type protein and the biological significance of phosphorylation remained to be elucidated. Phosphorylation is enhanced by LXR ligands. Expression of some but not all established LXR target genes is increased in macrophages expressing mutant LXR- α ^[88].

PPAR γ is the closely related nuclear receptor to LXR and shares a common role in macrophage cholesterol turn over. Both receptors shares some common features, such as they form heterodimers with RXR and their endogenous activators are oxidized lipid molecules i.e. oxidized fatty acids for PPAR γ and oxidized sterols for the LXR. Both are involved in the regulation of lipid metabolism in adipose tissue, macrophage and liver. Transplantation of PPAR γ null bone marrow into LDLR^{-/-} mice resulted in a significant increase in atherosclerosis^[89]. Further it was reported that TZDs inhibited the development of atherosclerosis in LDLR deficient male mice^[90], similar results were reported in the atherosclerotic model of apoE^{-/-} mice^[91]. All the above reported observations consider PPAR γ as an antiatherogenic molecule. PPAR γ expression has also been found in foam cells of atherosclerotic lesions and its expression could be increased with the oxidized LDL. PPAR γ enhances uptake of oxidized LDL but not native LDL^[92] by inducing the scavenger receptor CD36 leading to a vicious cycle of cholesterol loading and foam cell formation. In concordance with others, our studies suggest that PPAR γ and LXR- α regulate each other. PPAR γ activators have been found to enhance cholesterol efflux *via* the LXR-ABCA1 dependent pathway^[93]. In two independent studies from our laboratory with PPAR γ and LXR- α knock down cellular models using an siRNA approach it was found that both regulate expression of each other^[75,94]. So the existence of this transcriptional cascade predicts that alterations in one of the elements in the cascade will affect all others and the net effect on cholesterol levels in the cell depends on how the balance between influx and efflux changes. Thus cen-

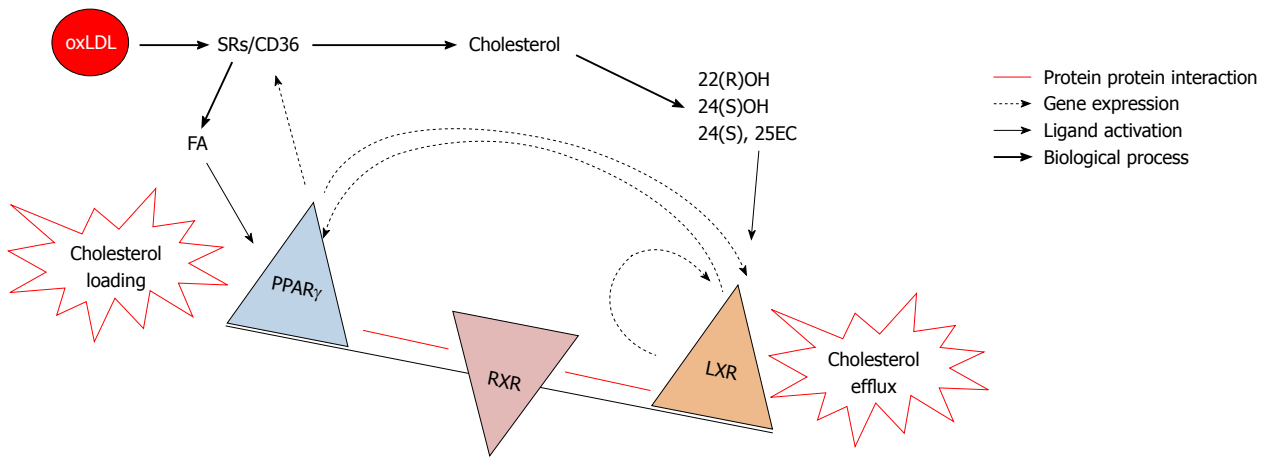


Figure 5 Decision maker: LXR vs PPAR γ .

tral activity of LXR- α determines if lipids are eliminated through cholesterol efflux towards HDL or accumulate and form foam cells from macrophages to induce lesion formation. This model highlights LXR- α molecule as a decision maker for atherosclerosis (Figure 5).

LXR- α AND CHD

From *in vitro* to *in vivo* model systems, it is now clear that LXR- α have the ability to regulate key processes i.e. lipid metabolism and inflammation, which are associated with the pathogenesis of atherosclerosis, making it a candidate molecule for the therapeutic intervention of the world's highest death causing disease. It has been shown that treatment with a synthetic LXR agonist GW3965 can reduce atherosclerotic lesion development in two mouse models (i.e. LDLR-/- and apoE-/- mice)^[95]. In addition to this, effectiveness of another synthetic agonist T-0901317 has also been reported^[96]. It is evident that anti-atherosclerotic actions of synthetic LXR agonists in murine models is to a large extent independent from changes in the plasma lipid profile which indicates that this effect is predominantly a consequence of direct action of LXR activators on the vascular wall. Consistent with this notion, synthetic LXR agonists were shown to stimulate ABCA1 and ABCG1 expression in the atherosclerotic lesions of both LDL receptor- and apoE-deficient mice^[95,96]. Subsequent experiments using bone marrow transplantation approaches provided direct evidence for a protective role of macrophage LXRs in atherosclerosis development. Tangirala *et al.*^[97] demonstrated that hematopoietic stem cell-specific LXR α/β deficiency aggravates atherosclerosis in both apoE and LDL receptor null mice. Another mechanism that could potentially contribute to the antiatherosclerotic action of LXR activators is their suppressing effect on macrophage inflammatory mediators production. Joseph *et al.*^[4] demonstrated that GW3965 and T0901317 inhibit expression of iNOS, cyclooxygenase-2 and interleukin-6 in macrophages subjected to bacterial infection or lipopolysaccharide stimulation. This inhibition depends on both LXR α and LXR β

and is mediated through suppression of the NF- κ B signaling. Anti-inflammatory action of LXR agonists has been confirmed *in vivo* in a model of contact dermatitis and in the aortas of atherosclerotic mice^[4].

In addition to stimulating reverse cholesterol transport and repressing inflammatory responses, LXR- α agonists may inhibit atherogenesis in several ways. For example T0901317 and GW3965 suppress platelet derived growth factor or insulin induced proliferation of vascular smooth muscle cells by inhibiting cell cycle progression from G₁ to S phase^[98]. The proliferation of smooth muscle cell plays an important role in growth of atherosclerotic plaques. It has been found that LXR agonist reduces the expression of cyclin D1 and cyclin A, which stimulates cyclin dependent kinases. Further TF is abundant in the lipid rich core of atherosclerotic plaques, and plaque rupture induces coagulation by exposing TF to circulating blood. It has been reported that synthetic LXR agonist attenuates LPS, TNF- α , and IL-1 β induced TF expression in murine and human macrophages^[71]. GW3965 has also been found to reduce cytokine induced synthesis and secretion of MMP-9, which is responsible for degrading the fibrous cap of atherosclerotic plaques and contributes to plaque rupturing^[70]. A recent report shows that^[99] LXR agonist attenuates the stimulatory effect of homocysteine (Hcy) on immunoglobulin production by B-lymphocyte, by attenuating reactive oxygen species (ROS) and NF- κ B activity. Although this study relates to a specific function of immune cells, these results are of great interest, taking into consideration the important role of the immune response in atherogenesis as well as an involvement of ROS and Hcy in cardiovascular pathology.

The immunohistochemical study indicated that LXR- α is highly expressed in macrophages present in human atherosclerotic lesions^[100]. Our previous study also demonstrated that blood cellular LXR- α gene expression was higher in normolipidemic and hyperlipidemic CHD groups as compared to their corresponding groups^[101], suggesting nature's protection against the development of CHD. In concordance with others^[102] findings from our

laboratory also stated that the statins, which are the best drug of choice for the treatment of CHD, exert their effect *via* the upregulation of LXR- α gene expression^[103]. Vitamin C also shares a common pathway for the upregulation of LXR- α ^[103]. The naturally occurring polyphenol resveratrol has been associated with the beneficial effects of red wine consumption on cardiovascular disease and has been shown to inhibit atherosclerosis in animal models. Resveratrol was shown to regulate the expression of LXR- α in human macrophages, which could be a possible molecular explanation for the beneficial effects of polyphenols^[104]. However our recent study revealed the existence of deregulated LXR- α transcriptome and a paradoxical relationship between blood cellular LXR- α mRNA expression and the severity of coronary occlusion, which was explained by the presence of three critical mutations in the ligand binding domain comprising Asp324, Pro327 and Arg328, responsible for the inability of this domain to interact with its natural ligands leading thereby to a deregulated LXR- α transcriptome^[35]. Keeping in view the importance of LXR- α signaling in CHD our study has raised the thrust for the search for alternative ligands for the restoration of deregulated LXR- α genomics in subjects suffering from CHD.

In summary, from cellular to animal model systems LXR activators have been found to be atheroprotective in nature by promoting reverse cholesterol transport, suppressing inflammatory processes and inhibiting vascular smooth muscle cell proliferation. Further higher expression of LXRs in the atherosclerotic lesions as well as in the peripheral blood mononuclear cells of CHD patients shows nature's protection against the development of CHD.

CONCLUSION

LXR- α signaling pathway has an established role in atherosclerosis and possesses all the features of a candidate molecule for the treatment of CHD by its ability to modulate genes involved not only in the cellular lipid homeostasis but also in the control of inflammatory processes. The great challenge lies in the development of the alternative ligands for LXR- α having the pharmaceutical values, which can activate them and can modulate genes selectively by means of promoting reverse cholesterol transport, inhibiting inflammatory processes and avoiding its lipogenic activities. In addition to this futuristic research, safe and effective LXR- α therapeutics will make LXR- α as an extraordinary target for the treatment of CHD.

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Regulatory role of mitochondria in oxidative stress and atherosclerosis

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Abstract

Mitochondrial physiology and biogenesis play a crucial role in the initiation and progression of cardiovascular disease following oxidative stress-induced damage such as atherosclerosis (AST). Dysfunctional mitochondria caused by an increase in mitochondrial reactive oxygen species (ROS) production, accumulation of mitochondrial DNA damage, and respiratory chain deficiency induces death of endothelial/smooth muscle cells and favors plaque formation/rupture *via* the regulation of mitochondrial biogenesis-related genes such as peroxisome proliferator-activated receptor γ coactivator (PGC-1), although more detailed mechanisms still need further study. Based on the effect of healthy mitochondria produced by mitochondrial biogenesis on decreasing ROS-mediated cell death and the recent finding that the regulation of PGC-1 involves mitochondrial fusion-related protein (mitofusin), we thus infer the regulatory role of mitochondrial fusion/fission balance in AST pathophysiology. In this review, the first section discusses the possible association between AST-inducing factors and the molecular regulatory

mechanisms of mitochondrial biogenesis and dynamics, and explains the role of mitochondria-dependent regulation in cell apoptosis during AST development. Furthermore, nitric oxide has the Janus-faced effect by protecting vascular damage caused by AST while being a reactive nitrogen species (RNS) which act together with ROS to damage cells. Therefore, in the second section we discuss mitochondrial ATP-sensitive K^+ channels, which regulate mitochondrial ion transport to maintain mitochondrial physiology, involved in the regulation of ROS/RNS production and their influence on AST/cardiovascular diseases (CVD). Through this review, we can further appreciate the multi-regulatory functions of the mitochondria involved in AST development. The understanding of these related mechanisms will benefit drug development in treating AST/CVD through targeted biofunctions of mitochondria.

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Key words: Apoptosis; Atherosclerosis; ATP-sensitive K^+ channels; Free radical; Mitochondrial biogenesis

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INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in many industrialized societies. Atherosclerosis (AST) is the major risk factor for the development of CVD based on arterial endothelial dysfunction.

tion caused by the impairment of endothelial dependent dilation^[1,2]. AST is a chronic inflammatory syndrome with a predilection for sites within the walls of arteries. Common factors involved in the mechanism of AST include endothelial cells, vascular smooth muscle cells, macrophages, platelets, and protein substances such as low-density lipoproteins (LDL), growth factors, and cytokines. In the initial phase of AST development, LDL or other pro-inflammatory proteins stimulate endothelial cell expression of adhesion factors, chemokines, and growth factors, which attract circulating monocytes to the inflamed endothelial cells, and then the monocytes enter the intima where macrophage colony-stimulating factor induces monocyte-macrophage differentiation. The macrophages through the uptake of oxidatively modified LDL and cholesterol lead to foam cell formation, augmenting early lesion development. Meanwhile, T lymphocyte activation, smooth muscle cell proliferation and migration, and extracellular matrix deposition occur in the vascular intima. These deposits interact with molecules in the intima and lead to necrosis, causing the formation of fibrous plaques which contain a core of lipid-laden cells and a fibrous cap formed by smooth muscle cells and matrix. Slow progressive enlargement of plaques may eventually cause them to rupture and with blood flow produce stenosis or closure of the lumen of other arteries. In addition, as a result of plaque rupture, platelet activation may lead to thrombosis, in which a thrombus obstructs blood flow, further resulting in clinically acute CVD or stroke. AST is a dynamic process, exhibiting constant changes in size and composition of the blood vessels. This is known as remodeling of the vessel^[3,4]. Two major forms of remodeling may progress within the affected artery. One is thickening and constrictive geometric remodeling in which the lumen of the affected artery becomes narrowed. The other is expansive remodeling in which the artery tends to preserve the lumen by expanding the vessel wall outward. These two remodeling responses by the vessels have been reported to depend on a variety of endogenous and environmental factors^[3]. They can vary from vessel to vessel, with age and gender, and are modulated by known cardiovascular risk factors.

Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and the extremely reactive hydroxyl radical, are risk factors for AST associated with lipid and protein oxidation in the vascular wall. ROS formation triggers a cascade of events such as oxidative modification of LDL, inflammation, cellular apoptosis and endothelium injury. ROS are produced by phagocytes and non-phagocytic cells through different mechanisms. The enzymes for ROS production in non-phagocytic cells such as vascular cells include xanthine oxidase, cytochrome P-450, uncoupled nitric oxide synthase (NOS), and NAD(P)H oxidase. Once ROS are produced, they play an important role in cell signaling^[5]. They can also attack vital cell components such as polyunsaturated fatty acids, proteins, nucleic acids, and even carbohydrates. These reactions can alter cell membrane properties such as fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, and DNA damage eventually leading to cell apoptosis. Mitochondria are the primary intracellular source of ROS,

as they generate huge numbers of oxidative-reduction reactions and use massive amounts of oxygen. Overproduction of mitochondrial ROS (mtROS) not only leads to mitochondrial DNA (mtDNA) damage and mutation but also triggers proapoptotic protein release into the cytosol and could further impair cell viability. Therefore, mitochondrial redox balance is the key regulator of cell survival and death by controlling the accumulation of mtDNA mutations and net production of ROS. Aging is the driving force in a cycle that begins with age-related changes in the blood vessels including arterial thickening and stiffening, and thus can increase susceptibility to CVD^[6]. Besides aging, the severity of mtDNA damage is higher in aortic samples from atherosclerotic patients than in non-atherosclerotic aortic samples from age-matched transplant donors^[7].

Net production of mtROS is another crucial mechanism by which mitochondria are thought to contribute to a variety of pathologies, for instance, CVD, aging, ischemia/reperfusion and neurodegenerative diseases. The balance of mitochondrial redox reactions is coordinated with the processes and components involved in ROS synthesis, antioxidant defense and ROS release^[8-11]. The role of mitochondria in ROS metabolism had been widely investigated in oxidative stress^[12,13]. In 1997, one of the drugs involved in mitochondrial activation, diazoxide, was found to have strong cardioprotective activity. Diazoxide is a selective agonist of mitochondrial ATP-sensitive K⁺ (mitoK_{ATP}) channels. Several studies have strongly implied that mitoK_{ATP} channel opening benefited the decline of excessive mtROS preservation *via* a mitochondrial permeability transition pore (PTP)-mediated leak in ischemia/reperfusion-induced injury of the heart or kidney. However, the related role of mitoK_{ATP} channels is still controversial^[11,14,15]. Although most studies focused on the effect of mitoK_{ATP} channels on ischemia/reperfusion^[11,16], these channels are associated with known complex disorders induced by cardiovascular risk factors including hypertension^[17], diabetes, and insulin resistance^[18].

Oxidative stress plays an important role in the initial occurrence of CVD and has been previously discussed^[19-21]. However, the molecular mechanisms responsible for mitochondria-mediated disease processes are not clear especially in mitochondrial physiology and in the balance of mitochondrial redox reactions during oxidative stress. Thus, the present study will review the mitochondrial biogenesis-mediated molecular mechanisms by which atherosclerotic risk factors could lead to mitochondrial dysfunction and subsequent vascular impairment. We will discuss (1) the role of mitochondrial dynamics in mitochondrial dysfunction and apoptosis; (2) the regulatory mechanism of mitochondrial biogenesis under AST-related stress; and (3) ROS/reactive nitrogen species (RNS)-mediated oxidative stress on mitochondrial biogenesis and physiology.

MITOCHONDRIAL DYNAMICS IN MITOCHONDRIAL DYSFUNCTION AND APOPTOSIS

Mitochondria are dynamic organelles in eukaryotic cells

and their heterogeneous morphology which results from equilibrium is dominated by the opposing processes of fission and fusion. The dynamic nature of mitochondria is a concept that includes the movement of mitochondria along the cytoskeleton, the regulation of mitochondrial architecture (morphology and distribution), and connectivity mediated by tethering and fusion/fission events^[22]. This dynamic network is essential to maintain normal mitochondrial functions and participates in fundamental processes, including development, metabolic efficiency, apoptosis, and aging^[23]. In recent years, at least four dynamin-related GTPase family proteins, including mitofusin 1 (MFN1), MFN2, optic atrophy 1 (OPA1), and dynamin-related protein 1 (DRP1), have been found to play significant roles in the regulation of mitochondrial fusion/fission^[24]. Mitochondrial fusion controls mitochondrial morphology and is constituted by a multi-step process including fusion in the outer and inner membranes^[24]. In outer membrane fusion, MFN1 and MFN2 which are located in the outer membrane help tether adjacent mitochondria by forming homo- and heterodimers. Furthermore, in mitochondrial inner membranes, OPA1 *via* oligomer formation is responsible for the formation of the cristae junction, ultimately resulting in mixing of matrix contents^[25]. In contrast, mitochondrial fission is regulated by DRP1 in the outer membrane^[26]. Fusion/fission balance plays a role in determining the fate of a depolarized mitochondrion. Mitochondrial fusion serves to maintain several mitochondrial functions or prevent stress-induced mitochondrial dysfunction. Besides the known fact that intact mitochondria can complement a damaged unit^[23], it was later proved that the process can be regulated by a physiological mechanism; fission leading to selective fusion segregates dysfunctional mitochondria and allows autophagy to eliminate them^[24]. Mitochondrial fission occurs when mitochondria are dysfunctional, probably caused by OPA1 cleavage^[27]. However, preventing fission leads to mitochondrial dysfunction and loss of mtDNA^[28]. Excessive mitochondrial fission and a lack of fusion results in breakdown of the mitochondrial network, loss of mtDNA, respiratory defects and an increase in ROS^[29]. Additionally, the mitochondrial dynamics in cells with oxidative stress was further explored based on the importance of mitochondrial fission and fusion processes on cell survival^[30,31]. Generally, in healthy cells the dynamic balance between mitochondrial fusion and fission maintains mitochondrial functions, but when cells are affected by excessive oxidative damage that results in apoptosis, the rate of mitochondrial fission outpaces that of fusion and stimulates fission protein DRP1-mediated release of cytochrome *c*. However, recently Tondera *et al.*^[31] offered a new concept for the distinct role of mitochondrial fusion in cells responding to stress using low levels of toxic agents such as cycloheximide, UV irradiation or actinomycin D. They found that cells subjected to low levels of stress-induced mitochondrial hyperfusion (SIMH), called the SIMH pathway, showed reduced mitochondrial fragmentation which occurs in full-blown apoptosis-induced cells. The mitochondria in SIMH fused into a closed network to confer resistance to further stress although

eventually cells may succumb to apoptosis. Further supporting evidence showed that the mitochondrial inner membrane protein, *stomatin*-like protein 2 (SLP-2), is up-regulated under conditions of mitochondrial stress leading to increased protein turnover^[32]. It is known that SLP-2 is significantly involved in the regulation of the stability of specific mitochondrial proteins. In HeLa cells, depletion of SLP-2 results in increased proteolysis of prohibitins and of subunits of the respiratory chain complexes I and IV^[32]. Moreover, SLP-2 is essential in SIMH and is dependent on the correlation of OPA1 and MFN1^[31].

The significance of cell death in AST has been demonstrated and clearly identified over the past few years in the related fields of key cellular, cytokine and molecular regulators within atherosclerotic lesions. The major consequence of AST in humans is mainly caused by apoptosis of vascular smooth muscle cells, endothelial cells and macrophages, possibly leading to promotion of plaque growth and pro-coagulation and induction of rupture^[2,33]. Mitochondrial dynamics plays a unique role in cell apoptosis. An imbalance of mitochondrial fusion/fission results in excessive fragmentation or tubulation, with pathological consequences^[30]. In addition to the influences of mitochondrial dysfunction and energetic deficiency on cell death, mitochondrial fission-related proteins also appear to participate in apoptosis and proteins associated with the regulation of apoptosis have been shown to affect mitochondrial ultra-structure^[34]. Mitochondrial-mediated apoptosis upstream of caspase activation is regulated by two proapoptotic *Bcl-2* family members, *Bax* and *Bak*, whose activation is through regulating mitochondrial outer membrane permeability (MOMP). Apoptosis-associated MOMP is known to require *Bax* and/or *Bak* which reside on the mitochondrial outer membrane or translocate there in response to proapoptotic stimuli^[35,36]. *Bax* then coalesces into foci with *Drp1*, mitofusins, and *Bak*, leading to mitochondrial division and release of cytochrome *c*^[37]. When cytochrome *c* is released from the mitochondrial space between the inner and outer mitochondrial membranes to the cytosol, it binds apoptotic protease-activating factor 1 (APAF1), activating the assembly of the apoptosome that activates caspase 9 and subsequently the effectors caspase-3 and caspase-7^[38]. The mitochondrial-triggered cytochrome *c* release is activated by an increase in fission. Blocking this mitochondrial fission inhibits cytochrome *c* release and delays cell death. Thus, mitochondrial fragmentation appears to be universally associated with apoptosis. However, excessive mitochondrial fragmentation is not linked to apoptosis such as in the cases of viral infection and stimulation by uncoupling agents^[39]. Mitochondrial fragmentation is not required to induce cell death as shown in the studies of Sheridan *et al.*^[40] and Breckenridge *et al.*^[41]. Likewise, caspase inhibitors did not affect the apoptotic fragmentation of mitochondria^[34].

Several studies have conclusively shown that activation of *Bax* and/or *Bak* rapidly leads to mitochondrial fragmentation^[42-45]. The phenomenon seems to be unrelated to MOMP and cytochrome *c* release^[46]. Interestingly, the study by Karbowski *et al.*^[47] found that, in *Bax/Bak* double-knockout cells, mitochondrial fusion was reduced and

inhibition of Drp1 activity did not promote elongation of mitochondria. The emerging role for the *Bcl-2 family* members, *Bax* and *Bak*, in regulation of mitochondrial dynamics was discussed recently^[48]. These authors proposed that the role of Bcl-2 proteins in mitochondrial morphogenesis is functionally distinct from their role in apoptosis^[48]. Bax and Bak can promote mitochondrial fusion by directly binding with Mfn1 and Mfn2 in normal cells and do not interfere with the normal fission process^[37,47]. These results further explain the foregoing discussion on the non-dependent interaction between mitochondrial fragmentation and apoptosis. However, the detailed mechanism is unclear, for instance, how does the apoptotic-stimuli-induced Bax translocate to mitochondria without changing the Bax-Mfn2 interaction.

REGULATORY MECHANISM OF MITOCHONDRIAL BIOGENESIS UNDER AST-RELATED STRESS

Of the causes which induce AST, oxidative stress is the most frequent and major cause, and AST is further induced by common risk factors. Under most conditions, the rates of cellular ROS formation and elimination are balanced through mitochondrial and cellular mechanisms that sense relative ROS levels. However, a chronic imbalance in redox homeostasis contributes to various chronic diseases, especially AST, which is an inflammatory disease associated with the oxidation of lipids and proteins in the vascular wall^[21,49]. Besides the influence of ROS/RNS formation, cytokines and chemoattractant chemokines recruit mononuclear leukocytes, and the migration, growth, and activation of the multiple cell types within atherosclerotic lesions are factors in the chronic inflammatory and fibroproliferative response central to AST. The role of oxidative damage in the cardiovascular risk factor-induced mitochondrial dysfunction in AST, such as aging, diabetes, dyslipidemia, hypertension homocysteinemia and cigarette smoking, has been reviewed in several literature studies^[50-53]. However, the regulatory mechanisms of those factors in mitochondrial functions are still unclear, especially in pathophysiological processes. Therefore, in this section we will discuss the related regulatory mechanisms of mitochondrial physiology (biogenesis and fusion/fission) during the development of AST and CVD.

Poly(ADP-ribose) polymerase-1 (PARP-1), the most abundant isoform of the PARP enzyme family, is a nuclear enzyme involved in modulating chromatin structure, regulating gene transcription, and sensing and repairing DNA damage^[54]. The protein is 116-kDa in size and is composed of three main domains: the N-terminal DNA-binding domain containing two zinc fingers, the automodification domain, and the C-terminal catalytic domain^[54]. When PARP-1 is activated by single- and double-stranded DNA breaks, it forms homodimers and catalyzes the cleavage of NAD⁺ into nicotinamide and ADP-ribose to form long branches of ADP-ribose polymers on the glutamic acid residues of a number of target proteins including PARP-1 itself. Although PARP-1 is essential for repairing stress-in-

duced DNA damage, the massive DNA damage and over-activation of PARP-1 leading to irreversible cellular energy failure (with depletion of cellular NAD⁺ and its precursor ATP)^[55,56], resulting in PARP-1 hyperactivation-induced necrosis has been implicated in several pathophysiological conditions^[57-60]. Moreover, PARP-1 is also involved in regulation of the apoptotic cascade by mediating the translocation of apoptosis inducing factor (AIF) from the mitochondria to the nucleus in oxidatively injured cells^[54,61]. The poly(ADP-ribose) polymer induced by PARP-1 activation had been demonstrated to be an AIF-releasing factor responsible for PARP-1-dependent cell death^[62,63]. The role of PARP-1 in cardiovascular disorders has been widely discussed in recent years^[59,64,65]. The major cause is dependent on PARP-1-regulated transcriptional levels of various proteins implicated in inflammation such as inducible NOS (iNOS), intercellular adhesion molecule-1, cyclooxygenase 2 and major histocompatibility complex Class II^[66]. Moreover, PARP-1 acts as the co-activator to regulate nuclear factor kappa-light-chain-enhancer of activated B cells and nuclear factor κ B (NF- κ B)-mediated transcription in the control of inflammatory cytokine/chemokine gene expression^[66,67]. It is well-known that induction of cytokine/chemokine receptor-activated NF- κ B signaling from activated endothelial cells and inflammatory cells in the vascular intima is an essential step in the development and progression of AST^[68]. In addition, the formation of atherosclerotic plaques has been demonstrated to require PARP-1 by the use of chronic PARP inhibitor and genetic PARP1 deletion on plaque formation in AST-prone apolipoprotein E knockout mice^[57]. Inhibition or genetic deletion of PARP-1 diminished the expression of inducible iNOS, vascular cell adhesion molecule-1, and P- and E-selectin^[57]. Furthermore, pharmacological inhibition of PARP-1 reduced the number of plaque inflammatory cells and decreased features of plaque vulnerability^[69]. In addition to AST, activation and overexpression of PARP-1 were also found in circulating mononuclear cells from unstable angina patients and had promoted PARP-1/NF- κ B/DNA complex formation, leading to enhanced expression of TNF- α and interleukin-6^[59]. In summary, the key pathophysiological roles of PARP-1 are shown in a simplified scheme in Figure 1. PARP-1 hyperactivation induced by excessive DNA damage during oxidative damage has various roles in promoting AST/CVD development *via* the regulation of AIF-dependent cell death, inflammatory gene transcription, cell adhesion molecules and the secretion of inflammatory cytokines.

PARP-1 *via* nuclear-mitochondrial crosstalk modulates the mitochondria-to-nucleus translocation of AIF which contributes to DNA fragmentation as previously described. However, the distinct role of PARP-1 in the regulation of mitochondrial biogenesis and fusion/fission has been explored recently. Hossain *et al*^[70] showed that nuclear respiratory factor 1 can directly interact with PARP-1 by co-purifying the PARP-1-DNA-PK Ku80-Ku70 topoisomerase II β -containing protein complex. They suggested that NRF-1 can activate PARP1-mediated transcription by enhancing recruitment of RNA polymerase II and/or by using enzymatic activities of

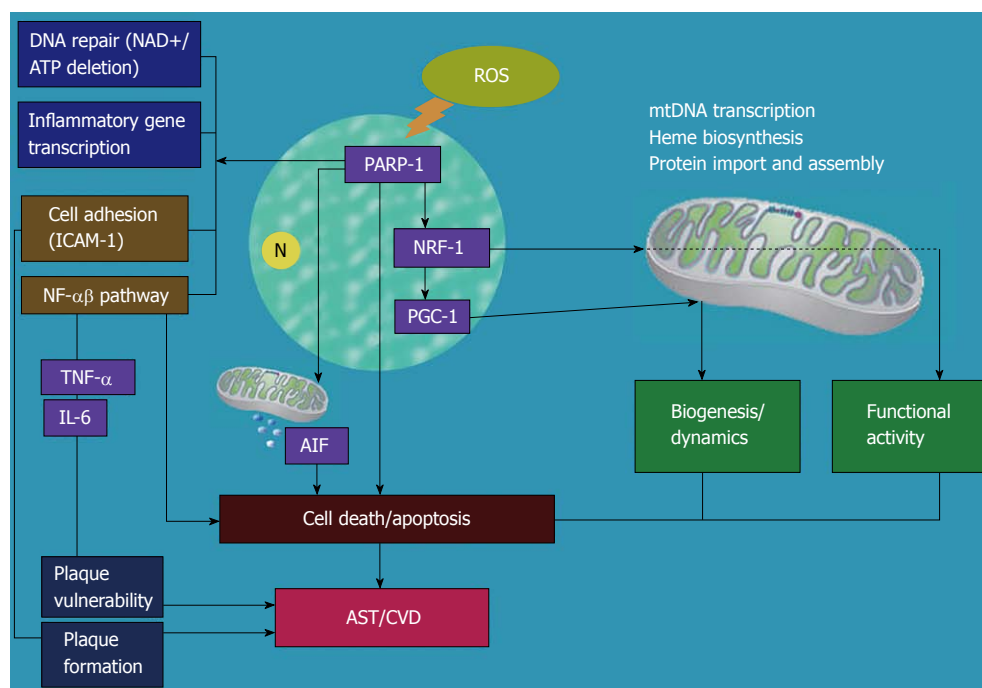


Figure 1 The molecular regulation of nuclear and mitochondrial cross-talk signaling on ROS-triggered AST/VCD development. ROS activate PARP-1 and cause AST primarily by increasing endothelial and smooth muscle cell death (mitochondria-dependent or not) and triggering inflammatory reactions. In addition, proinflammatory cytokines and chemokines induce plaque formation and plaque vulnerability that leads to deterioration in the later stages of AST. However, PARP-1 can also participate in NRF-1 regulation or indirectly activate PGC-1 to improve mitochondrial biofunctions such as biogenesis and fusion. This mechanism defends against ROS-induced progression of AST by inhibiting mitochondrial-mediated apoptosis and against the occurrence of AST at an early stage. ROS: Reactive oxygen species; PARP-1: Poly(ADP-ribose) polymerase-1; NRF-1: Nuclear respiratory factor 1; PGC-1: Peroxisome proliferator-activated receptor γ coactivator-1; mtDNA: Mitochondrial DNA; AIF: Apoptosis-inducing-factor; NF- κ B: Nuclear factor κ -light-chain-enhancer of activated B cells; TNF- α : Tumor necrosis factor- α ; IL-6: Interleukin-6; AST: Atherosclerosis; CVD: Cardiovascular diseases; N: Nucleus.

the PARP-1·DNA-PK·Ku80·Ku70·Topo II β -containing complex. PARP-1 can also PARylate the DNA-binding domain of NRF-1 and negatively regulate the NRF-1·PARP-1 interaction^[70]. Mitochondria contain their own genetic system consisting of a circular double-stranded DNA (mtDNA), which is responsible for the synthesis of 13 essential subunits of the inner membrane complexes of the respiratory apparatus. Because of myriad mitochondrial functions based on the limited coding capacity of mtDNA, most protein subunits consisting of five inner membrane complexes of the electron transport chain and oxidative phosphorylation system rely heavily on the expression of the nuclear encoding genes to maintain all the mitochondrial functions. NRF-1 is one of the transcriptional factors for nuclear-coded genes and has been linked to the expression of many genes required for mitochondrial respiratory functions^[71]. The nuclear control of mitochondrial functions by NRF-1 was shown to include the regulation of mitochondrial translation, heme biosynthesis, and mtDNA transcription/replication^[72].

Recent findings suggest that NRF-1 is also involved in the expression of key components of the protein import and assembly machinery in mitochondria and plays a broader role in orchestrating events in mitochondrial biogenesis beyond the regulation of respiratory chain-related genes^[63,72,73]. NRF-1 is involved in the nucleomitochondrial interactions through interplay with the peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-

1 α), one of the transcriptional coactivator family (PGC-1 α , PGC-1 β , and PGC-1-related co-activator), to induce mitochondrial biogenesis^[74,75]. PGC-1 α is mainly involved in the regulation of gluconeogenesis and has the potential to integrate the activities of a diverse collection of transcription factors implicated in the expression and function of the mitochondrial oxidative machinery^[72]. Its transcriptional level is highly dependent on the increase in energy expenditure caused by cold exposure (in brown adipose tissue), fasting (in liver) or exercise (in muscle)^[72,76]. These expressions differ somewhat from that of PGC-1 β . For instance, PGC-1 β expression occurs in distinct tissues and is unaffected by physiological processes as described above^[76,77]. Even if PGC-1 α and PGC-1 β are functionally distinct in the regulation of mitochondrial biogenesis and uncoupling proteins^[71,72,78,79], they both participate in the induction of mitochondrial biogenesis by utilizing NRF-1 or other transcription factors^[72]. Thus, overexpression of either PGC-1 α or PGC-1 β contributes to an improvement in oxidative phosphorylation in cells with mtDNA mutation^[80]. We further proposed that the interaction of NRF-1 and PGC-1 α somehow affects the mitochondrial dynamics in maintaining proper mitochondrial morphology and activity during mitochondrial dysfunction. The hypothesis of mitochondrial biogenesis involving the balance of mitochondrial fission-fusion is further supported by the finding of Soriano *et al.*^[81], who showed that PGC-1 α can induce Mfn2 transcription and regulate mitochon-

drial activity which depends on correct Mfn2 expression. Moreover, in 2008, Liesa *et al.*^[82] demonstrated that PGC-1 β increases mitochondrial fusion using a multi-approach strategy that combined Mfn2 knockout cells, PGC-1 β -overexpressing muscle cells and PGC-1 β -ablated mice. They showed that reduced mitochondrial size observed in transcriptional regulator PGC-1 β knockout mice was associated with a selective reduction in Mfn2 expression, and that PGC-1 β increases mitochondrial fusion and elongates mitochondrial tubules by enhancement of *Mfn2* gene transcription through co-activated nuclear receptor estrogen related receptor α . Thus, these results prove that transcriptional regulators such as NRF-1 not only affect mitochondrial biofunction but also shift the balance between mitochondrial fusion and fission events through coordination of the PGC family to selectively control gene expressions (Figure 1). Here, we try to explain the distinct role of PPAR-1 during AST/CVD development. As shown in Figure 1, in the early stage of ROS-induced oxidative stress, cells can protect themselves from oxidative damage by activation of PARP-1-related signaling to support mitochondrial biofunctions and prevent mitochondrial-mediated cell apoptosis. When this is not enough to sustain the extensive ROS production, PARP-1 plays the opposite role by promoting the progression of AST/CVD. This phenomenon explains why mitochondrial hyperfusion is observed in cells suffering lower oxidative stress^[31,32], as previously described.

ROS/RNS-MEDIATED OXIDATIVE STRESS ON MITOCHONDRIAL BIOGENESIS AND PHYSIOLOGY

ROS-mediated mitochondrial biogenesis was demonstrated through the upregulation and actions of NRF-1^[83]. Moreover, ROS can also induce mitochondrial biogenesis through direct regulation of PGC-1 α transcription^[84,85]. Several reports link the expression of PGC-1 α to exercise-induced mitochondrial biogenesis in skeletal muscle^[74,85,86], but few have demonstrated the role of PGC-1 α -mediated mitochondrial biogenesis in AST/CVD. As we know, the production of ROS is a crucial trigger for induction and progression of AST. ROS induce vascular inflammation *via* the proinflammatory cytokine/NF- κ B pathway. Kim *et al.*^[87] found that overexpression of the PGC-1 α gene in human aortic smooth muscle and endothelial cells leads to a significant reduction in intracellular and mtROS production as well as NAD(P)H oxidase activity which is induced by TNF- α . These findings implied that PGC-1 α -mediated mitochondrial biogenesis in oxidatively injured cells seems to offer a good source of “healthy mitochondria” which detoxify mtROS by a large antioxidant defense system containing numerous redox enzymes of the electron-transport chain decreasing net ROS production. Thus, stimulation of PGC-1 α expression in the vasculature benefits the prevention of AST development. Recently, another regulatory mechanism of PGC-1 α -mediated apoptosis of endothelial cells was shown to support this inference. Adenoviral overexpression of PGC-1 α prevented linoleic acid-induced

increases in ROS generation and cell apoptosis in human aortic endothelial cells by increasing fatty acid oxidation, decreasing cytosolic fat metabolites and increasing ATP/ADP translocase activity^[88]. Fatty acid oxidation affects metabolic and CVD by the regulation of adiponectin (adipocytokine), a circulating plasma protein secreted by adipocytes^[89,90]. Adiponectin has insulin-sensitizing metabolic effects and vascular protective properties^[91], and helps inhibit the inflammatory reaction induced by oxidative stress or TNF- α in endothelial cells^[90]. In addition, adiponectin binds to the walls of catheter-injured vessels^[92], and inhibits the expression of several TNF- α -induced adhesion molecules and monocyte adhesion in cultured endothelial cells^[93]. Adiponectin also inhibits the production of CXC receptor 3 chemokine ligands in macrophages and reduces T-lymphocyte recruitment in atherogenesis^[94]. Ouedraogo *et al.*^[95] showed that adiponectin suppresses excess ROS production induced by high-glucose conditions *via* a cAMP/PKA-dependent pathway. This implied a possible connection between mitochondrial biogenesis and adiponectin-mediated vascular protection. Indeed, it was demonstrated that adiponectin and adiponectin receptor 1 (AdipoR1) regulated mitochondrial functions by PGC-1 α ^[96]. The binding of adiponectin and AdipoR1 increased PGC-1 α expression and activity by Ca²⁺ signaling and by AMP-activated protein kinase and Sirtuin type 1, leading to increased mitochondrial biogenesis^[96]. However, it still needs to be proved whether the regulatory mechanism observed in skeletal muscles to explain the causes of mitochondrial dysfunction and insulin resistance in diabetes, exists in endothelial cells and vascular smooth muscle cells to regulate AST/CVD.

Adiponectin has vascular actions which directly stimulate production of nitric oxide (NO) in endothelial cells through phosphatidylinositol 3-kinase-dependent pathways involving phosphorylation of endothelial NOS (eNOS)^[75,97]. It is known that NO derived from eNOS acts as a maintenance factor for vascular structure integrity and has a variety of antiatherogenic effects in physiological conditions. Moreover, endothelial dysfunction which occurs early in the development of AST is characterized by a loss of NO bioactivity. Although NO plays a protective role in AST, it is still a reactive radical (RNS) that leads to nitrosative stress when excessive RNS generation in a biological system can not be neutralized. NO reacts strongly to ROS and produces peroxynitrite anion, which is a potent oxidizing agent capable of causing oxidative damage^[50]. Thus, the Janus-faced role of eNOS in vascular disease is as a superoxide-producing enzyme especially in ROS-induced cardiovascular pathophysiology^[98]. Under a high level of oxidative stress, the peroxynitrite anion from the strong interaction between NADPH oxidase-derived superoxide and eNOS-derived NO oxidizes tetrahydrobiopterin (BH4), an essential cofactor of eNOS, leading to a reduction in NO bioactivity and a further increase in ROS production (Figure 2). ROS are the major factors in the vascular inflammatory reaction leading to the development of atherosclerotic lesions. Mitochondrial dysfunction which induces the AST process not only depends on imbalanced mitochondrial dynamics as mentioned previously,

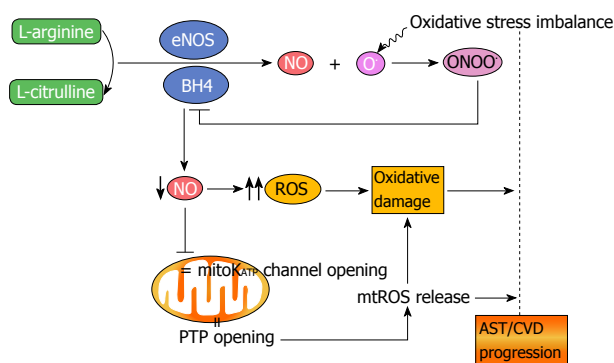


Figure 2 Proposed facilitation of AST progression via the opening of mitoK_{ATP} channels. Decreased NO production by eNOS and increased expression of ROS induce AST. Excessive ROS with affinity for NO bind with it to produce the peroxynitrite anion (ONOO⁻) which inhibits tetrahydrobiopterin (BH₄), an essential cofactor of eNOS, leading to further reduction of NO. Significantly reduced NO not only increases ROS production, but also induces the opening of mitoK_{ATP} channels, followed by activation of the permeability transition pore (PTP), leading to mtROS release and AST development. mtROS: Mitochondrial ROS; NO: Nitric oxide; mitoK_{ATP}: Mitochondrial ATP-sensitive K⁺; eNOS: Endothelial nitric oxide synthase.

but also relies on the excess release of mtROS related to the important sources and targets of ROS in mitochondria^[10,99]. Several studies have demonstrated that activation of mitoK_{ATP} channels decreases ROS generation by preventing mtROS release and plays a protective role in the heart against ischemia-reperfusion injury^[11,16,100] and mimics ischemic preconditioning^[101]. The mitoK_{ATP} channel opening improves the recovery of contractility and reduces myocardial infarct size in the reperfused heart^[102,103] and could be associated with the mechanisms of mitochondrial calcium uptake prevention, the decrease in mtROS release and blockage of PTP^[11,104]. As described previously, MOMP is a key participant in the machinery that controls mitochondrial fate and, consequently, cell fate. The opening of PTP causes cell death by increasing the release of cytochrome *c* and AIF^[46] and triggering mtROS release *via* PTP^[11]. Moreover, cytochrome *c*-catalyzed peroxidation of cardiolipin, a mitochondrial phospholipid, has been shown to reduce the binding of cytochrome *c* to the mitochondrial inner membrane and facilitate permeability of the outer membrane, which leads to mitochondrial dysfunction as well as the initial phase of the apoptotic process^[105]. Recently, the role of mitoK_{ATP} channel opening was further revealed by melatonin-mediated protection against heart ischemia-reperfusion injury^[106]. The regulatory mechanism is related to inhibition of cardiolipin peroxidation in mitochondria and prevention of mitochondrial permeability transition and cytochrome *c* release^[107]. Melatonin seems to have antiapoptotic actions in normal cells *via* the regulation of PTP and cytochrome *c* release^[108,109] but the opposite regulation has been observed in different cell types such as tumor cells^[110]. This suggests that considerable variability exists in the permeabilization of the outer membrane among different cell types treated with melatonin. Therefore, the role of mitoK_{ATP} channels in the regulation of cytochrome *c* release and ROS-induced cell death needs to be considered carefully.

Although the role of the mitoK_{ATP} channel-mediated mechanism in heart injury is mostly defined, its role in the occurrence and development of CVD such as AST is still unknown. Wajima *et al.*^[111] found that intravenous administration of BH₄ has a cardioprotective effect in rats with myocardial infarction following ischemia/reperfusion, and its protective effect appeared to be involved in the opening of mitoK_{ATP} channels through increased NO production. NO, as previously mentioned is one of the *antiatherogenic* factors, so we can hypothesize that NO protection in excessive ROS-induced progression of AST could result from the opening of mitoK_{ATP} channels to trigger the blockage of PTP. This mechanism explains that a reduction in NO leads to a significant increase in ROS and induces oxidative injury in AST. Besides eNOS-dependent regulation, the reduction in NO inhibits mitoK_{ATP} channel opening, and further increases oxidative stress caused by mtROS release (Figure 2). NO also benefits cardioprotection *via* the stimulation of mitoK_{ATP} channel opening^[112]. Although the activation of mitoK_{ATP} channels by NO was observed in cardiomyocytes^[111,113], the existence of a similar mechanism in endothelial or smooth muscle cells requires further research^[108].

CONCLUSION

ROS-mediated cell apoptosis/death plays a major role in AST/CVD. Mitochondria are thought to augment intracellular oxidative damage by generating ROS and releasing cytochrome *c* and other *pro-apoptotic* proteins such as AIF. However, several defense systems in mitochondria which regulate ROS metabolism and outer membrane permeability simultaneously determine cell fate. During ROS-induced progression of AST/CVD, mitochondrial physiology including biogenesis, fusion/fission and mitoK_{ATP} channel-mediated ROS release participates in related regulation *via* hyperactivation of PARP-1. PARP-1 not only promotes mitochondria-dependent cell death in injured cells and thus facilitates the development of AST/CVD, it also induces the defense functions in cells at the same time *via* the induction of mitochondrial biogenesis, hyperfusion and biofunctions in an early stage of AST/CVD progression. On the other hand, in terms of ROS/RNS imbalance as a major factor in AST/CVD occurrence, the role of mitoK_{ATP} channels in ROS metabolism was further elucidated and showed that extensive ROS induce a dramatic decline in eNOS-mediated NO production, which inhibits the opening of mitoK_{ATP} channels, leading to mtROS release from PTP during AST. Thus, ROS-induced progression of AST is facilitated. This review will benefit our understanding of the resistance mechanism in mitochondria against oxidative stress and offers distinct opinions on mitochondrial physiology in the progression of AST/CVD.

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Profound thrombocytopenia induced by clopidogrel with a prior history of long-term safe administration

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Abstract

Clopidogrel has shown an excellent safety, tolerability and efficacy ever since its marketing. However, here we report a rare case with profound thrombocytopenia following clopidogrel administration previously safely exposed to this same drug. This reminds us that thrombocytopenia might be induced by clopidogrel even with a prior, safe history of long-term administration.

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Key words: Clopidogrel; Thrombocytopenia; Coronary artery disease; Stent

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INTRODUCTION

Clopidogrel, in combination with aspirin, is commonly used for the prevention of thrombosis in patients who have received coronary artery stents^[1]. Moreover, it has shown an excellent safety, tolerability and efficacy since its marketing^[2]. However, there have been cases of clopidogrel-associated serious adverse effects, including cases of thrombocytopenia and severe allergic reaction (including cutaneous reactions and angioedema)^[3,4]. Here we report a rare case of thrombocytopenia induced by clopidogrel in a patient with a prior history of safe, long-term administration.

CASE REPORT

A 58-year-old male had a super-lateral wall myocardial infarction without revascularization on February 20, 2000. On November 26, 2002, he presented with an exertional angina pectoris, and coronary angiography showed a 95% stenosis of the left anterior descending artery (LAD), which was treated with a 3.5 mm × 24 mm of sirolimus-eluting Cypher coronary stent (Cordis Corporation, Warren, New Jersey) after an oral loading dose of 300 mg of clopidogrel (Co. sanofi Winthrop Industrie, France). After stenting, he received a daily dose of 75 mg of clopidogrel until November 30, 2003, with persistently normal laboratory tests, including platelet count, and without any other side effects.

He was referred to our department due to an acute super-lateral wall myocardial reinfarction on December

17, 2008. Emergent coronary angiography showed a total occlusion of the intermediate branch (IB), a 90% stenosis of the right coronary artery (RCA) and a patent LAD, with the Cypher stent. Thus, the lesion in the IB was treated with a balloon angioplasty, and, subsequently, a 2.5 mm \times 18 mm Vision coronary stent (Guidant Corporation, USA) was implanted. At the time of stenting, coagulation function assays and the complete blood count were normal, with a platelet count of $207 \times 10^9/L$, a leukocyte count of $8.08 \times 10^9/L$ and a neutrophil percentage of 59%. To prevent the thrombosis of the stent, an oral loading dose of 300 mg of aspirin and 300 mg of clopidogrel (Co. Sanofi Winthrop Industrie, France) were administered before the procedure, followed by a daily dose of 300 mg aspirin and 75 mg of clopidogrel. In addition, heparin and tirofiban were administered during and after the procedure, respectively. Additional drugs included atenolol, atorvastatin and nitrates. About 8 h after the intervention, the patient presented with a mid-high fever and the platelet count sharply dropped to $4 \times 10^9/L$ without obvious bleeding and/or thrombosis. The platelet count was immediately repeated taking blood samples with citrate as the anticoagulant, in order to rule out EDTA-dependent pseudothrombocytopenia. However, values were confirmed at $4-6 \times 10^9/L$, accompanied by an increasing leukocyte count ($14.31-15.81 \times 10^9/L$) and a neutrophil percentage of 95.7%-96.4%. Tirofiban was discontinued, while clopidogrel was maintained, due to its safe history of administration. Intravenous dexamethasone and promethazine, followed by platelet transfusion and high doses of human immunoglobulin, were started. The platelet count persisted very low, with a nadir count of $2 \times 10^9/L$ and minor bleedings in the urinary and alimentary tract. Blood bilirubin and coagulation function assays remained normal and a bone marrow biopsy showed an actively proliferating marrow with increased megakaryocytes but no platelet-generated megakaryocytes. Thus, on the 4th d, clopidogrel was discontinued and replaced by cilostazol, 200 mg per day, orally. Then, the platelet count stably increased to $> 50 \times 10^9/L$, without using platelet transfusions and/or human immunoglobulins. The highest platelet count, i.e. $319 \times 10^9/L$, was reached on the 12th d, together with a leukocyte count of $8.63 \times 10^9/L$ and neutrophil percentage of 68.5%. Occult blood tests, carried out on urine and stool samples, were both negative again. The diagnosis of clopidogrel-associated thrombocytopenia was highly suspected and aspirin was subsequently resumed. The patient was discharged with a normal complete blood count, and placed on oral aspirin, 100 mg daily, and cilostazol, 200 mg daily, combined with atenolol, atorvastatin and nitrates.

The patient received another stenting procedure in his RCA and aspirin, 300 mg, plus cilostazol, 200 mg, per day, orally, on February 16, 2009. Four months later, the patient again complained of chest pain induced by strenuous exertion, and x-ray computerized tomography of the coronary arteries showed a 50% in-stent restenosis

of both the IB and the RCA. In view of further, possible coronary intervention, and based on the previous safe history of clopidogrel administration, and after careful consideration, the patient took half a tablet of clopidogrel (37.5 mg). Just an hour later, he reported chills, followed by fever and, several hours later, he developed gingival bleeding, hemorrhagic spots and petechial purpura of both upper extremities. The platelet count dropped from $213 \times 10^9/L$ to $6 \times 10^9/L$, while the leukocyte count increased from $6.45 \times 10^9/L$ to $9.06 \times 10^9/L$ and the neutrophil percentage from 57.9% to 93% within 6 h. The platelet count recovered promptly in 3 d upon clopidogrel withdrawal. No major adverse event occurred.

DISCUSSION

Thrombocytopenia is a rare but dangerous adverse effect of clopidogrel, encompassing thrombotic thrombocytopenic purpura (TTP), isolated thrombocytopenia and autoimmune thrombocytopenia^[5-7]. The features of the present case included: (1) a 1-year safe history of clopidogrel administration; (2) two events of clopidogrel-associated thrombocytopenia without evidence of TTP; and (3) rapid drops of the platelet count accompanied by typical chills and fever. As of today, there have been reported only five cases of clopidogrel-induced thrombocytopenia without evidence of TTP. However, none of them had a safe history of clopidogrel administration and none had allergic manifestations at the time the platelet count dropped. The clinical manifestations reported in our case could not be completely explained by the clopidogrel-associated thrombocytopenia reported before^[8]. Certainly, both episodes of thrombocytopenia were induced by clopidogrel, and the mechanism may be due to a rare but severe allergic reaction upon repeated exposure to clopidogrel. This suggests that clopidogrel may induce some sensitizing antibodies at the time of the first treatment. When clopidogrel was administered for the second time, these antibodies led to thrombocytopenia. This problem may be solved by desensitization, should the allergic mechanism be demonstrated to be dependent on an IgE type of allergic reaction^[9]. In this rare situation, clopidogrel can be effectively replaced, such as in our case, with cilostazol, an anti-platelet drug which is not of common use in the Western countries. Furthermore, the new generation anti-platelet drugs, such as ticagrelor and prasugrel, might also play a role^[10-12].

To the best of our knowledge, this is the first case of potential sensitization-associated thrombocytopenia brought about by clopidogrel. Thrombocytopenia always poses a dilemma in the management of patients, especially those in whom stents were recently placed. Additionally, nearly every antiplatelet agent might induce thrombocytopenia, and this makes more complicated to decide which drug one should discontinue first^[13]. The case we reported above reminds us that a rare, allergic thrombocytopenia might be induced by clopidogrel, even in patients with a prior history of safe, long-term administration.

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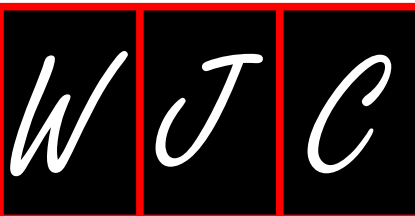
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Meetings

Events Calendar 2010

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January 17-21
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22nd Annual International
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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
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Symposium

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Multi-Modality Imaging

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Cardiac Problems in Pregnancy

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

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Scottsdale, United States
International Congress XXIII on
Endovascular Interventions

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Keystone, United States
Keystone Symposia: Cardiovascular
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- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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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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- 9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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- 11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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- 12 **Breedlove GK**, Schorheide AM. Adolescent pregnancy. 2nd ed. Wiczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

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- 14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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- 15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/EID/eid.htm>

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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Write as mean \pm SD or mean \pm SE.

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