Correlation of Vulnerable Coronary Plaques to Sudden Cardiac Events. Lessons from a Myocardial Infarction-prone Animal Model (the WHHLMI Rabbit)

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It is generally considered that coronary rupture-prone plaques play an important role in the onset of sudden cardiac events (acute coronary syndromes/sudden cardiac death). However, it is not clear which factors or stimuli are required to trigger plaque rupture and whether coronary plaques without occlusive thrombi can cause sudden cardiac events. To address these issues, recently, we developed a rabbit model of spontaneous myocardial infarction [the Watanabe heritable hyperlipidemic (WHHL) MI rabbit] and found that this model possessed several types of coronary plaques that are possibly correlated to sudden cardiac events. Although many of the coronary plaques of the WHHLMI rabbits appeared histologically to be rupture-prone in nature, true rupture was detected only in the few animals that died of MI. In addition, no occlusive thrombus was detected in any WHHLMI rabbit. These findings suggest that some additional stimuli play a definitive role in causing disruption of rupture-prone plaques and thrombosis. Nearly-occluded plaques caused by a luminal macrophage accumulation are the most common feature of WHHLMI rabbits, suggesting that they are responsible for sudden cardiac events. The WHHLMI rabbit could be a useful model for studying the mechanism(s) of plaque rupture and thrombogenesis if plaque rupture/thrombus formation is induced in the rupture-prone plaques of WHHLMI rabbits by administration of additional triggering factors, and could provide a novel means for developing new therapies. J Atheroscler Thromb, 2004; 11: 184–189.

Key words: Coronary atherosclerosis, Myocardial infarction, Vulnerable plaque, WHHLMI rabbit

Introduction

Sudden cardiac events (acute coronary syndromes and/or sudden cardiac death) are the major cause of death in developed countries. It has been reported that more than 19 million people worldwide suffer a sudden cardiac event every year (1–2). Studies over the past decade have provided many novel insights into culprit plaques responsible for sudden cardiac events.

Histopathological examinations of hearts from patients who died from sudden cardiac events have shown that in most cases, occlusive thrombus formation was caused or initiated by plaque rupture or erosion (3–5). In sudden cardiac events, plaque rupture is the most common cause of plaque complications.

Rupture-prone plaques, which are characterized by possessing an easily disrupted/fissured and occlusive thrombus, have a large lipid core and a thin fibromuscular cap accompanied by many macrophages and other inflammatory cells on or beneath the cap surface (6–8).
Immunohistochemical study has shown that macrophages accumulated in the plaque express high levels of matrix metalloproteinases and tissue factors (9). These enzymes are considered to make the fibrous cap fragile (7–9) because they can digest the extracellular matrix in the fibrous cap and subsequently lead to thrombus formation (7–9). Based on such studies, rupture-prone plaques have come to be considered a crucial trigger in the process of sudden cardiac events and also a target for clinical intervention. However, it is still difficult to predict when a rupture-prone plaque will disrupt since the additional physiological stimuli required to cause the event are unknown. The mechanisms of plaque rupture and subsequent occlusive thrombus formation are still not clear. In addition, it is also unclear whether plaques without an accompanying occlusive thrombus can cause sudden cardiac events. Recent studies have described plaques with a high probability of undergoing a rapid progression, in addition to thrombosis-prone plaques, as "vulnerable plaques", and suggested the importance of vulnerable blood (prone to thrombosis) and vulnerable myocardium (prone to arrhythmia) in the onset of sudden cardiac events (1, 2).

These proposals are thought-provoking but require verification. To address issues regarding the mechanism of plaque rupture and the onset of myocardial infarction and to prove the hypothesis proposed by Naghavi et al. (1, 2), suitable animal models are needed. Several genetically modified mouse models with hypercholesterolemia and atherosclerosis have been reported (10–12). However, histopathological findings of coronary atherosclerosis and subsequent myocardial lesions resembling those of humans have not been presented. None of the lesions of the left main coronary arteries presented in the reports (10–12) had the typical fibrous cap, and some of the lesions resembled the intimal thickening with fibrosis observed in the arterioles of humans and rabbits. In the heart, the development of fresh myocardial lesions is obscure.

Since lipoprotein metabolism in mice is largely different from that in humans (13, 14), a mouse model may not be suitable for studies examining the effects of hypolipidemic therapies on atherosclerosis or myocardial infarction (MI). In addition, the mouse is small in size, which may hamper its use in many surgical manipulations and therapeutic interventions. Therefore, there is a need to develop a relatively large animal model for the study of MI.

Recently, we developed an animal model in which MI develops spontaneously [the Watanabe heritable hyperlipidemic (WHHL) MI rabbit], through the selective breeding of coronary atherosclerosis-prone WHHL rabbits (15). WHHLMI rabbits suffer from a fatal MI and vulnerable plaques as defined by Naghavi et al. (1, 2). This model should open the way for studies of the mechanism(s) of plaque rupture, thrombus formation when plaque rupture/thrombus formation is induced in the rupture-prone plaques of WHHLMI rabbits by administration of additional triggering factors, and the onset of subsequent sudden cardiac events. In addition, this animal model could be useful for studying the development of new therapies for these diseases and instruments for the detection of vulnerable plaques. In this review, we report the characteristics of the WHHLMI rabbit and the use of this model in the study of vulnerable plaques and sudden cardiac events.

**A Newly Developed Animal Model of MI (WHHLMI Rabbits)**

Recently, we developed an animal model, designated the WHHLMI rabbit, in which sudden cardiac events occur spontaneously without any artificial treatment (15). The cumulative incidence of fatal sudden cardiac events up to the age of 35 months was 97%. In the hearts of these rabbits, representative findings of MI were observed (Fig. 1). This animal model was developed from the coronary atherosclerosis-prone WHHL rabbit, which suffers from coronary atherosclerosis (16), by selective breeding. Originally, the WHHL rabbit was established by Watanabe (17) as a model of human familial hypercholesterolemia (FH) due to an LDL receptor deficiency (18). We examined WHHLMI rabbits that died suddenly and found ischemic myocardial lesions, coronary arteries totally occluded with atheromatous plaques, and representative changes in electrocardiograms during the development of MI (15).

![Fig. 1. Representative myocardial infarction of a female WHHLMI rabbit aged 18 months. Myocardial fibrosis was observed in the subendocardial region of the left ventricle wall, and the postal region of the right wall. Occluded plaques were observed in the circumflex artery and septal artery. (Azan-Mallory staining)](image-url)
Correlation of vulnerable plaques to the onset of sudden cardiac events

Although the WHHLMI rabbits suffered from MI and the coronary plaques in many of the rabbits appeared to be rupture-prone (Fig. 2A), only a few plaques were accompanied by features of rupture or fissure (small rupture), while matrix metalloproteinases-positive macrophages were frequently present in these coronary plaques, as previously observed in aortic plaques (9). This finding was initially unexpected and surprising. We postulated that rupture-prone coronary plaques are important in the onset of sudden cardiac events, but this finding suggests that the direct triggers for plaque rupture play a definitive role in the disruption of rupture-prone plaques. Several candidates for risk factors have been proposed, such as sympathetic hyperactivity, inflammation of the plaque, dysfunction or denudation of arterial endothelial cells, and others, in addition to general risk factors for atherogenesis such as hypercholesterolemia, cigarette smoking, hypertension, diabetes, and social stress.

In the WHHLMI rabbits, no occlusive thrombus was detected in the coronary arteries, but many of the coronary arteries were occluded by an accumulation of a large number of macrophages at the plaque surface (Fig. 2B). Some of these plaques showed a loss of the arterial endothelium, infiltration of blood components, intra-plaque hemorrhage, or calcified nodules (15). In addition to rupture-prone plaques, these coronary plaques were probably the culprits behind the onset of sudden cardiac events in the WHHLMI rabbits. Recently, Naghavi et al. (1) recommended the term “vulnerable plaques” to identify plaques with a high probability of undergoing a rapid progression, in addition to all thrombosis-prone plaques. They also suggested that vulnerable plaques consist of seven types: rupture-prone plaques, ruptured/healing plaques, eroded plaques, plaques with intraplaque hemorrhage, plaques with calcified nodule, and critically stenotic plaques (above 90% stenosis). Therefore, the occlusive macrophage accumulation observed in many coronary plaques of the WHHLMI rabbits probably corresponded to the critically stenotic type of vulnerable plaque proposed by Naghavi et al. (1). Therefore, the findings of the coronary plaques in WHHLMI rabbits probably support the suggestion of Naghavi et al. (1).

Thrombogenesis and the onset of sudden cardiac events

In the onset of sudden cardiac events, thrombogenic blood is also important (2). An occlusive thrombus has frequently been observed in the coronary arteries of patients who died from sudden cardiac events. Therefore, thrombus formation at the coronary plaque surface is another important factor in the triggering of sudden cardiac events. In the WHHLMI rabbits, however, no occlusive thrombus was detected in the coronary arteries, despite a disappearance of the endothelial cells at the plaque surface or the exposure of macrophages to the blood circulation. In the WHHL rabbits, which did not suffer from sudden fatal cardiac events, the administration of Russell’s viper venom (150 μg/kg of body weight, intraperitoneally) in combination with angiotensin II (30 μg/kg of body weight, intravenously) induced coronary thrombosis and subsequent acute MI (19). These results indicated that drastic conditions were required to induce coronary thrombosis in the WHHL rabbits. Regarding the coagulation of blood in the WHHL rabbits, the results of studies were controversial. In comparison to normal rabbits, the WHHL rabbits had significantly high levels of plasma clotting factors (20); their aggregative responses

Fig. 2. Vulnerable plaques observed in the circumflex arteries of WHHLMI rabbits. (A) A rupture-prone plaque characterized by a thin fibromuscular cap with a large lipid core and macrophages accumulated beneath. (B) An occluded coronary plaque with macrophages accumulated at the surface. (Elastic van Gieson staining)
to platelet activating factor, adenosine diphosphate, and collagen were reduced despite an aggregative response similar to that of normal rabbits following thrombin stimulation (21); and their coagulation time was not reduced in terms of prothrombin time, activated partial thromboplastin time, or thrombin time (22). In addition, macrophages in atheromatous plaques of WHHL rabbits expressed tissue factor (9), which is a potent promoter of thrombus formation, and many macrophages at the surface of atheromatous plaques were exposed to the blood circulation due to the disappearance of arterial endothelial cells in WHHLMI rabbits (15). However, no coronary thrombus was detected (15). These findings suggest that factors other than tissue factor and the disappearance of endothelial cells play an important role in thrombus formation in WHHL/WHHLMI rabbits. Karnicki et al. (23) demonstrated that the role assigned to lesion-bound tissue factor was not physically realistic in a porcine model and that blood-borne factors must have a major role in the propagation of thrombi. Therefore, more detailed studies are needed to examine the thrombogenicity of WHHLMI rabbits.

Correlation of myocardial conditions to the onset of sudden cardiac events

In the hearts of relatively young WHHLMI rabbits, excised within 30 min after death, no myocardial lesions were detected under a light microscope although the coronary arteries were almost completely occluded by the accumulation of a large number of macrophages at the plaque surface. However, transmission electron microscopy showed fresh ischemic changes in the nuclei and mitochondria of the myocardial cells (24). Therefore, these young WHHLMI rabbits probably died from the first ischemic heart attack. The death may have been caused by acute MI or fatal arrhythmias due to myocardial ischemia. In the ischemic myocardium without prior atherosclerosis-derived myocardial damage, autonomic nerve activity has a significant role in modifying the clinical outcome of coronary occlusion (2). Studies have demonstrated that strong afferent stimuli from the ischemic myocardium may impair the arterial baroreflex and lead to hemodynamic instability (25), and sympathetic hyperactivity leads to life-threatening ventricular tachyarrhythmias (26). On the other hand, aged WHHLMI rabbits had fresh myocardial lesions characterized by hyperemia, infiltration of inflammatory cells, and eosinophilic degeneration of myocardial cells, in combination with old myocardial lesions, fibrosis and/or myocardial scars (15). These aged WHHLMI rabbits probably survived the first ischemic heart attack and the myocardial lesions probably worsened with repeated ischemic damage to the base of the almost completely occluded coronary plaques. These two types of myocardial lesions suggest extensive inter-individual variation in the type and severity of autonomic reactions during the early phase of abrupt coronary occlusion (2), in addition to influences of the location of the occluded plaque (proximal or distal), speed of arterial occlusion (sudden or gradual), and other factors. The correlation of autonomic nerve activity to sudden cardiac events needs to be examined in WHHLMI rabbits. Furthermore, in the onset of sudden cardiac events in humans, an ischemic vulnerable myocardium with prior atherosclerosis-derived myocardial damage, an old or new myocardial infarction, inflammation, and/or fibrosis potentially increase a patient’s vulnerability to arrhythmia and sudden death (2). With the development of more effective treatments for acute coronary syndromes, more patients are now surviving an acute event, but some develop heart failure or ischemic cardiomyopathy later due to the potential for fatal arrhythmia (2). Therefore, the findings in the aged WHHLMI rabbits may resemble those in patients who have survived a first sudden cardiac event.

WHHLMI Rabbits vs Patients with MI

Stehbens and Martin reported the histopathological findings of an FH patient (27). They demonstrated several differences in coronary atherosclerosis between FH and non-FH. First, coronary lesions in FH patients are characterized by foam cells, which lead to severe stenosis, whereas in non-FH patients, the lesions are usually fibromuscular and accompanied by calcification. In addition, plaque rupture and occlusive thrombosis are basically rare in FH patients but frequent in non-FH patients; furthermore, myocardial lesions are circumferential as subendocardial fibrosis in FH patients but regional as fresh and massive transmural infarct in non-FH patients. Therefore, the onset of myocardial lesions is probably due to mainly pure foam cell-rich coronary lesions in FH patients, but due to mainly plaque rupture/erosion and consequent occlusive thrombi in non-FH patients. The findings of the WHHLMI rabbits closely resemble those of human homozygous FH but are different from those of non-FH patients, except that typical rupture-prone plaques were observed in WHHLMI rabbits. The findings on the coronary plaques and myocardial lesions of WHHLMI rabbits suggest that the mechanisms of ischemic damage are somewhat different from those of general human acute coronary syndromes in the formation of occlusive thrombi in the coronary arteries. However, it is difficult to conclude that these differences are due to the species. There are several other differences between WHHLMI rabbits and humans. WHHLMI rabbits have a low-risk existence, i.e., they are given a healthy diet low in cholesterol and rich in plant matter, and a stress-free environment in addition to no smoking. These conditions may suppress plaque rupture and thrombosis. In the future, we intend to modify the current WHHLMI
model and create human-like MI lesions by introducing genes or environmental factors that will trigger plaque rupture and subsequent occlusive thrombi.

**Future Uses of WHHLMI Rabbits in Studies of Sudden Cardiac Events**

As mentioned above, the final definitive triggers for plaque disruption or occlusive thrombus formation are unknown. In future studies, we hope to identify these triggers and elucidate how they disrupt coronary rupture-prone plaques or induce thrombogenesis by administering various agents to WHHLMI rabbits. WHHLMI rabbits may be useful for studying the influence of autonomic nerve activity on the onset of sudden cardiac events in the ischemic myocardium, with or without prior atherosclerosis-derived myocardial damage because of the inter-individual variation in the development of sudden cardiac events in WHHLMI rabbits. In addition, aged WHHLMI rabbits, which show fresh myocardial lesions accompanied by old myocardial lesions, may be useful for developing more effective treatments for survivors of a first heart attack.

Recently, genetic engineering technology has enabled us to develop transgenic rabbits (28). The transfer of genes related to plaque rupture or thrombus formation to WHHLMI rabbits may well provide a breakthrough in the study of vulnerable plaques, vulnerable blood, and vulnerable myocardium. Comparing the expression of matrix metalloproteinases, interferon-γ, or factors related to arterial contraction in plaque rupture; blood-borne factors, PAI-1, or other clotting factors in thrombosis; and factors related to autonomic nerve activity in vulnerable myocardium. Compared to transgenic mice, transgenic WHHLMI rabbits are more suitable for studies of atherosclerosis and sudden cardiac events because of a lipoprotein metabolism similar to that of humans, and their larger body size.

Finally, WHHLMI rabbits should be useful in the development of more effective compounds for the suppression of the formation of vulnerable plaques and the onset of sudden cardiac events.

**Acknowledgments:** This work was supported in part by a grant from the Sankyo Company. We thank Mr. Toshiaki Tamura for breeding the WHHLMI rabbits.

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