Thrombosis Research 169 (2018) 64–72

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Review Article

Murine models of vascular endothelial injury: Techniques and pathophysiology

Yue Wu, Sheng-an Su, Yao Xie, Jian Shen, Wei Zhu*, Meixiang Xiang*

Cardiovascular Key Lab of Zhejiang Province, Department of Cardiology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hang Zhou 310009, Zhejiang Province, China

ARTICLE INFO

Keywords:
Vascular endothelial injury model
Pharmacological intervention
Surgery
Genetic manipulation

ABSTRACT

Vascular endothelial injury (VEI) triggers pathological processes in various cardiovascular diseases, such as coronary heart disease and hypertension. To further elucidate the in vivo pathological mechanisms of VEI, many animal models have been established. For the easiness of genetic manipulation and feeding, murine models become most commonly applied for investigating VEI. Subsequently, countless valuable information concerning pathogenesis has been obtained and therapeutic strategies for VEI have been developed. This review will highlight some typical murine VEI models from the perspectives of pharmacological intervention, surgery and genetic manipulation. The techniques, pathophysiology, advantages, disadvantages and the experimental purpose of each model will also be discussed.

1. Introduction

Endothelial cells (ECs) refer to the innermost monolayer of flattened epithelium of heart, blood vessels and lymphatic vessels, serving as both mechanical and biological barrier of blood flow [1]. ECs not only provide a smooth surface for blood flow but also play a pivotal role in maintaining vascular permeability, regulating vascular tone and secreting vasoactive cytokines such as prostaglandin (PG), nitric oxide (NO) and endothelin (ET) [2,3]. Loss of ECs induced by specific gene deletion such as *LKB1* [4] and *PPAR-δ* [5] leads to vascular dysfunction which is regarded as an early event of atherosclerosis (AS) [6], hypertension [6] and diabetes mellitus [7,8]. Moreover, dysfunction of the endothelium is also involved in metabolic syndrome and dyslipidemia [9], which may contribute to the pathophysiology of obesity [10] and hyperhomocysteinemia [11]. Thus, ECs are indispensable for maintaining vascular homeostasis.

VEI can be induced by various pathogenic factors (Table 1). To elucidate the mechanisms and potential therapeutic targets of endothelial injury, different animal models, including murine, rabbit [12,13] and swine [14] have been built up and modified, simulating different cardiovascular diseases in human. Among all animals, murine are the most widely adopted due to their uncomparable advantages such as small size, easy manipulation, short breeding time and relative ease for care [15]. Although numerous murine models of vascular injury have been successfully built up, only few literatures focus on the underlying mechanisms. With advanced bio-technology, transgenic mice are employed to further analyze specific genes associated with VEI. For example, the apolipoprotein E-deficient mouse ($Apoe^{-/-}$) [16,17] and the LDL receptor-deficient mouse ($Ldlr^{-/-}$) [18], have been widely accepted for studying atherogenesis. The present review aimed to summarize the updated information about murine VEI models from three following aspects: pharmacological models, surgical models and genetic manipulation models. Detailed comparisons are also included between these commonly used models as shown in Table 2. Thus, our review will provide an overview about the various murine VEI models where endothelial cell injury serves as the initial step for different cardiovascular diseases such as hypercholesterolemia, diabetes, hyperhomocysteinemia and hypertension. However, due to the length limitation, the causative relationship between the various human disease and VEI model and their detailed molecular mechanism will not be covered.

2. Pharmacological murine VEI models

2.1. Oxidized low-density lipoprotein (ox-LDL) induced VEI model

The formation of oxidized low-density lipoprotein (ox-LDL) is regarded as the early stage of atherosclerotic lesions, followed by

* Corresponding authors at: Department of Cardiology, Second Affiliated Hospital Zhejiang University School of Medicine, 88 Jiefang Road, Hangzhou 310009, Zhejiang, China.
E-mail addresses: weizhu65@zju.edu.cn (W. Zhu), xiangmx@zju.edu.cn (M. Xiang).

https://doi.org/10.1016/j.thromres.2018.07.014
Received 14 March 2018; Received in revised form 8 June 2018; Accepted 8 July 2018
Available online 11 July 2018

Table 1
The pathogenic factors of endothelial injury.

Life style	Obesity, smoking, peristalsis reduction, mental pressure
Pathological status	hypertension, advanced age, coronary artery disease, peripheral arterial disease, chronic cardiac insufficiency, diabetes mellitus, etc.
Relative molecular and metabolism	ROS, H_2O_2, inflammatory chemokines (TNF-α, INF-β, IL, etc.), vasoactive peptides (Ang II, ox-LDL, Hcy, etc.), adhesion molecules (ICAM-1, VCAM-1, P-selectin, etc.), AGEs, etc.
Others	Hemodynamics, shear stress, ischemia-reperfusion

Abbreviation: AGEs: advanced glycation end products; Ang II: angiotensin II; Hcy: homocysteine; H_2O_2: hydrogen peroxide; ICAM-1: intercellular cell adhesion molecule 1; IL: interleukin; INF-β: interferon-β; ox-LDL: oxidized low-density lipoprotein; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-α; VCAM-1: vascular cell adhesion molecule 1.

Table 2
Comparison of different VEI models in murine.

Injury type	Stimulus factor and approach	VECs integrity	Medial injury	Modeling difficulties	Relative vascular diseases
Pharmacological models	ox-LDL	Yes	No	△	Thrombosis, Coronary artery disease, AS
	Hcy	Yes	No	△	Hyperhomocysteinaemia, Thrombosis, Stroke, AS, Coronary heart disease, Peripheral artery stenosis
	AGEs	Yes	No	△	Hyperglycemia, AS, Diabetes mellitus, Coronary artery disease
	Ang II	Yes	No	△	AS, Vascular calcification, Vascular senescence, Hypertension, HFpEF
	LPS	Yes	No	△	Sepsis, Hypotension, ARDS
	Nicotine	Yes	No	△	AS, Hypertension, Coronary artery disease
	Photochemical reaction	Yes	No	△	Thrombosis, Restenosis
Surgical models	Flexible wire	No	Yes	△△△	Thrombosis, Restenosis
	Balloon catheter	No	Yes	△△△	Restenosis, AS
	Vein graft	Maybe	Maybe	△△△△	Thrombosis, AS, Restenosis
	Electric current	No	Yes	△△	Thrombosis
	stent implantation	No	Yes	△△△	Restenosis, Thrombosis
	Air drying	No	Yes	△△	Restenosis, AS
Gene-manipulation models	$Apoe^{-/-}$	Yes	No	△	AS
	$Ldlr^{-/-}$	Yes	No	△	AS
	Cre-loxP system	No	No	△	AS, Hypertension, diabetes mellitus

Abbreviation: ARDS: Acute Respiratory Distress Syndrome; HFpEF: Heart Failure with preserved Ejection Fraction; △: degree of difficulty.

endothelial injury and homing of leukocytes [19]. LDL oxidation can be modified by bio-oxidation in vivo and chemical oxidation in vitro. For chemical oxidation of LDL, Cu^{2+} based LDL modification was commonly employed in vitro [20]. Jiang et al. [21] successfully built VEI model in male SD rats. Briefly, the rats were intravenously injected with 6 mg/kg of native LDL. Endothelial dysfunction was then induced by the elevation of asymmetric dimethylarginine (ADMA) levels. The mechanisms of ox-LDL induced VEI are potentially involved as followings: i) ox-LDL regulates the production of intracellular reactive oxygen species (ROS), subsequently activating nuclear factor-κB (NF-κB), which is a potent pro-apoptotic factor that can induce the apoptosis of VECs and lead to VEI [22,23]; ii) ox-LDL increases ADMA, reducing endothelium-dependent NO production, then it accelerates the development of VEI which eventually causes endothelial dysfunction and atherosclerosis [24]; iii) ox-LDL activates vascular endothelial cells (VECs) to express plasminogen activator inhibitor-1 (PAI-1), inhibiting the fibrinolytic system that can promote the necrosis of ECs, which consequently injures VECs [25].

The concentration of ox-LDL strongly correlate with the pathogenesis process of AS. Moreover, ox-LDL, as an independent risk factor for a series of acute and chronic inflammatory diseases, is a circulating marker of several cardiovascular diseases related with AS pathogenesis [26]. Augmented LDL oxidation has been observed in the diabetic states as well as during the development of hypertension. ox-LDL induced VEI model has a distinct mechanistic route of dyslipidemia which contributes to AS. Ox-LDL has numerous effects, but most of these effects were observed in vitro [27,28]. Therefore, it is urgent and ideal to induce an animal VEI model in vivo by manipulating ox-LDL from patients with AS. However, due to the barrier of the extracting techniques of ox-LDL from human serum, its in vivo application remained to be tested.

2.2. Advanced glycation end products (AGEs) induced VEI model

AGEs are the irreversible end products of anon-enzymatic glycation process. The expression of AGEs is increased upon hyperglycemia in diabetes [29]. The accumulation of AGEs in the vessel wall may perturb cell structure and function to trigger ECs damage and subsequently contribute to vascular dysfunction under hyperglycemia (Table 2). Therefore, AGEs are identified as pivotal pathologic factors in diabetes and cardiovascular diseases [30,31]. In the early 1990s, Vlassara et al. [32] built AGEs-induced VEI model by performing tail-vein injection in male Lewis rats. Rats were given tail-vein injections of 100 mg/kg per day exogenous AGEs for 2–4 weeks. Vascular tissue succeeded to exhibit pathological changes when the serum AGE reached to 99.6 ± 15.6 units/ml. AGEs-induced injury exhibits a conspicuous effect in murine model when the dosage is over 100 μg/ml [20]. Since then, the role of AGEs in the pathogenesis of VEI was confirmed in vivo [33]. The underlying mechanisms include: i) Intracellular AGEs formation enhances the glycation of protein, lowering the expression of oxygen in organs and endothelial NO synthase (eNOS). The down-regulation of NO level is then involved, which eventually damages the VECs [31]; ii) AGEs bind to specific cellular-receptors, including AGE-R1, AGE-R2 and receptor for advanced glycation end products (RAGE) [34] activate signal transduction cascade and up-regulate the expression of some pro-inflammatory genes such as Cdc42/Rac/NF-κB [35,36], and eventually impairing the VECs function; iii) AGEs can accumulate and interact with the extracellular matrix, altering the mechanic and structure properties of vessels, such as decreasing the elasticity and the size of vessel lumen, as well as increasing the stiffness and thickness of vessels [37–39]. Such pathological processes lead to the diminished vascular compliance and vascular dysfunction.

The AGEs-induced VEI model mimics the vascular abnormality under diabetes mellitus with long term of hyperglycemia. Therefore,