CHAPTER III
LITERATURE REVIEWS

3.1 Endothelial cell

3.1.1. Introduction

The vascular endothelium consists of specialized epithelial-like cells that line the luminal surface of all blood vessels and form the capillary networks. The endothelial cell (EC) layer in an adult human composes of approximately 1 to 6 x 10^{13} cells, weighs about 1 kg, and covers a surface area of around 1 to 7 m^2. ECs line vessels in every organ system. They regulate the flow of nutrient substances and produce several biologically active molecules such as nitric oxide. Endothelium also acts as a gate keeper through junctional proteins and numerous membrane-bound receptors for proteins (e.g. growth factors, coagulant, and anticoagulant proteins), lipid transporting particles (e.g. low-density lipoprotein (LDL)), metabolites (e.g. nitric oxide and serotonin), and hormones (e.g. endothelin-1) (1).

3.1.2 Endothelial cell (EC) function

Endothelial cells act as paracrine glands; they play an important role in the regulation of blood flow. These cells secrete and uptake vasoactive substances, which constrict and dilate specific vascular beds in response to stimuli. In addition, ECs also create an anticoagulant surface for regulating blood flow and preventing thrombus formation (Table 1). For example, thrombomodulin (TM) has been shown to be involved in regulating endothelial thromboresistance (14).

Table 3.1 Regulation of Hemostasis and Thrombosis by the Endothelium (14)

	Antithrombotic	Prothrombotic
Coagulation protein binding sites	**Thrombomodulin**	**Thrombin receptor** **Receptor for protein C/APC**
Products produced and/or stored by platelets	**PGI_2** **NO** **ATPase**	**vWF** **PAF** **Fibrinogen**
Vasomotor factors	**NO** **PGI_2**	**TxA_2** **Endothelin-1**

Thrombomodulin (TM)

TM (THBD, CD141, BDCA3, fetomodulin) acts as a multidomain integral membrane protein which constitutively expressed on the lumenal surface of vascular endothelial cells. TM serves as a binding site for thrombin and then activates the protein C (PC) through signaling cascade of TM. For example, it elicits potent anticoagulant effects, antifibrinolytic as well as anti-inflammatory properties (14).

Vasculogenesis

Vasculogenesis is a process of the new blood vessel formation from the pre-existing one by a *de novo* production of ECs. This process only occurs in the early embryogenesis. Vascular and hematopoietic tissues are developed together at the beginning after the implantation with the formation of blood islands within the primitive yolk sac (Figure 3.1). They comprise two cell types: (1) angioblastic cells responsible for forming the outer layer of ECs that covers the blood island; and (2) hematopoietic stem cells located in the inner cluster, which generates the first embryonic blood cells. The embryo also expresses several growth factors - particularly fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). The presence of tyrosine receptor kinases, VEGFR-1 (flk-1) and VEGFR-2 (flt-1) in mysenchymal cells are essential for the generation and proliferation of new ECs (Figure 3.1).

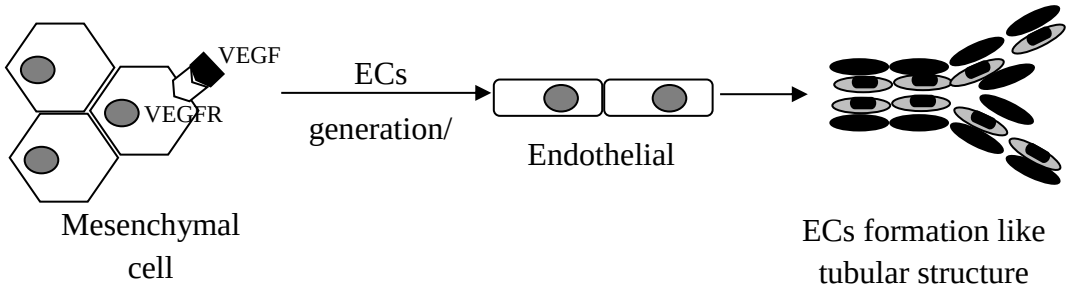

Figure 3.1 The formation of new vessels during vasculogenesis by VEGF stimulate mesenchymal cell to differentiate into endothelial cell generation and proliferation

Vasoregulation

The endothelium does not only provide a structural barrier between the circulations and surrounding tissues but also secretes mediators that influence vascular hemodynamics in the physiologic state. ECs play a major role in the regulation of blood pressure and blood flow by releasing vasodilators such as NO and prostacyclin (PGI_2) and vasoconstrictors such as endothelin (ET) and platelet-activating factor (PAF). NO is constitutively secreted by ECs, but its production is modulated by a number of exogenous chemicals and physical stimuli, whereas the other known mediators (PGI_2, ET, and PAF) are synthesized primarily in response to changes in the external environment (1).

3.1.3 Endothelial cell dysfunction

Endothelial dysfunction has been used to refer to several pathological conditions, including altered anticoagulant and anti-inflammatory properties of the endothelium, impaired modulation of vascular growth, as well as deregulated vascular remodeling. Normally, this term is referred to an impairment of endothelium-dependent vasorelaxation caused by a loss of nitric oxide (NO) bioactivity in the vessel wall. Several human studies reported that the traditional risk factors for atherosclerosis lead to the endothelial dysfunction. The impairment of endothelium-dependent vasodilation in the coronary circulation of humans has profound prognostic implications such as the prediction of adverse cardiovascular events and long-term outcome. The reduction of NO bioavailability in these conditions may be caused by decreased expression of the endothelial nitric oxide synthase (eNOS), altered cellular

signaling, lack of substrate or cofactors for eNOS expression and accelerated NO degradation by ROS (15). The endothelial dysfunction is caused by oxidative stress or steroid therapy which can assess from the component in thrombotic process such as soluble thrombomodulin (sTM). The sTM levels is elevated in children with idiopathic nephrotic syndrome (FENS) indicating endothelial dysfunction (16).

3.2 Nitric oxide

3.2.1 Introduction

Nitric oxide (NO) is a gaseous free radical that functions as an endogenous mediator of diverse biological effects in tissues. NO is generated from L-arginine, molecular oxygen, and NADPH. Enzyme nitric oxide synthase (NOS) catalyzes the change of L-arginine into L- citrulline and nitric oxide (NO). There are three forms of NOS: neuronal nitric oxide (nNOS or NOS-1), inducible NOS (iNOS or NOS-2), and endothelial NOS (eNOS or NOS-3). NOS-1 is mainly expressed in the brain. NOS-2 is found in macrophages and liver, and NOS-3 is found exclusively in the endothelium. However, certain tissues such as kidney, express all three isoforms. eNOS is activated by several agonists like acetylcholine (Ach), VEGF, estrogen, etc via calcium-dependent or calcium independent pathways. eNOS is activated through calcium-dependent pathway which induces by calcium-calmodulin complex (17). NO diffuses through plasma membrane and activates soluble guanylyl cyclase to form cGMP from GTP (Figure 3.2). Then cGMP activates the protein kinases resulting in the increased protein phosphorylation and altered cellular function. Like other intracellular messengers, cGMP levels are regulated by its release (transport or extrusion) from the cell and the rate of its degradation and inactivation. Some isoforms of PDE selectively hydrolyze cGMP or cAMP while other isoforms hydrolyze both cyclic nucleotides. (17). A small portion of the remaining intravascular NO may be oxidized to from nitrite, which is a major metabolite of NO. Red blood cells also act as a major intravascular storage pool of nitrite. Nitrite enters the red blood cells and reacts either with oxyhemoglobin (oxy Hb) to from nitrate and methemoglobin (met Hb) or with

deoxyhemoglobin (deoxy Hb) to from NO, nitrosylhemoglobin (NO-Hb), and NO adducts (RX-NO) (18).

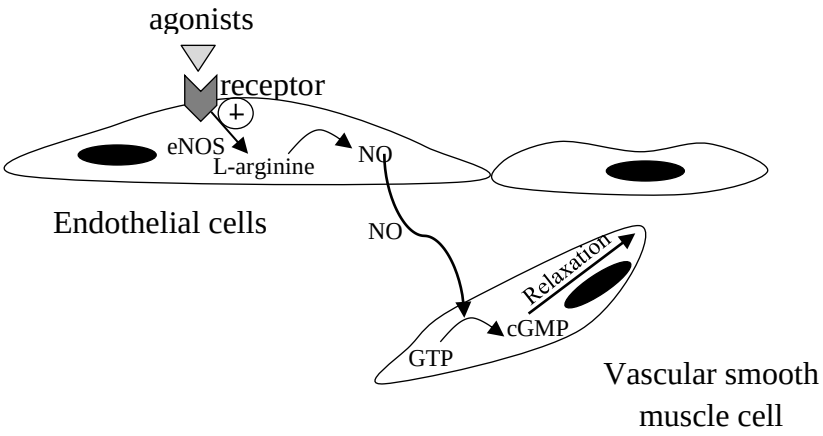

Figure 3.2 The schematic representation of the release of NO and of its effects on smooth muscle cells

3.2.2 Chemical biology of nitric oxide

Biological reactions of NO can be classified into two types. First, the direct effects occurring at low NO concentration are caused by the interaction of NO with its biological targets such as metal complexes and other radical species. Indirect effects of NO are involved with the reactive nitrogen species (RNS), which are derived under condition of relatively high NO concentration. These species including nitrite (NO_2) and nitrogen trioxide (N_2O_3) are products of the reaction of NO with either superoxide or molecular oxygen (19). Moreover, NO reacts with transition metals to alter their valence electron (20). For example, ferrous ion (Fe^{2+}) in the heme moiety of hemoglobin (Hb^{2+}) is converted to ferric iron (Fe^{3+}), resulting in the generation of methemoglobin (Hb^{3+}). NO rapidly reacts with superoxide anion to form $ONOO^-$. Moreover, NO also reacts with oxygen (O_2) to form the nitrite (NO_2^-) in the solution, whereas in the gas phase, the reaction of NO with O_2 produces the oxidizing gas NO_2. In addition, NO also reacts with the thiol group (RSH) to produce nitrosothiol (RS-NO) compounds. The NO reactive pathway is determined by biochemical characteristics of various components of the system (21)

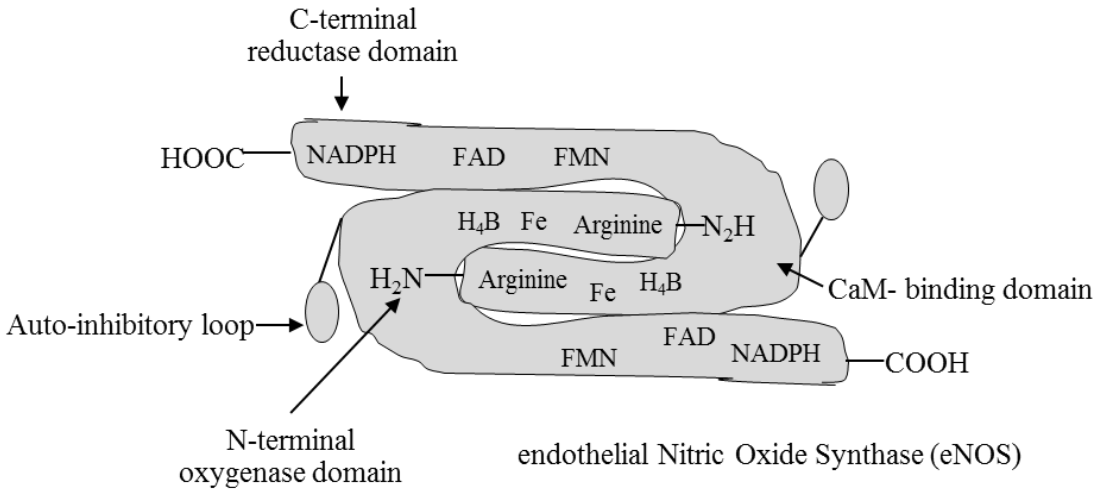

Figure 3.3 Basic structure of endothelial nitric oxide synthase (eNOS). eNOS composes 2 monomers which contains 3 major domains; 1) C-terminal reductase domain is located NADPH, FAD, and FMN. 2) N-terminal oxygenase domain contains heme (Fe), arginine, and tetrahydrobiopterin (BH_4). 3) Calmodulin (CaM)-binding domain. Adapted from reference (22).

3.2.3 Enothelial nitric oxide synthase (eNOS)

eNOS is synthesized as a monomers and dimer formation that composed of 3 domains, 1) C-terminal reductase domain binds nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD), 2) N-terminal oxygenase domain which is located a heme, and binding sites for arginine, and tetrahydrobiopterin 3) Calmodulin-binding domain. 3 domains are shown in Figure 3. eNOS is regulated by several mechanisms divided into calcium dependent pathway and calcium independent pathway. eNOS activity could be induced by several agonists like acetylcholine, bradykinin, β_2- adrenergic receptor, estrogen, sphingosine 1-phosphatase through intracellular elevation of calcium levels. However, there is a calcium Hemodynamic forces could stimulate eNOS activity also such as laminar shear stress and G protein through phosphoinoside 3-kinase (PI3K) and adenylate cyclase (AC) pathways leading to activate eNOS phosphorylation on serine 1177 for Akt and serine 633 for PKA.

3.2.4 Nitric oxide consumption

Mostly, the consumptive mechanism of NO depends on O_2, which is a key factor in determining the rate of NO consumption. The increase in oxygen levels is correlated to the increased NO consumption. Studies suggested that NO could regulate oxygen consumption through the inhibition of mitochondrial respiration. The concentration of NO and rates of NO consumption are determined by interacting of NO with red blood cells, ROS, as well as its intracellular metabolism. Another mechanism of NO consumption of NO is mediated by ROS through the interaction with peroxidases and Fenton-type reactions. It has been shown that H_2O_2 reacted with heme proteins and reduced NO concentration (19).

3.2.5 Nitric oxide and vascular effects

NOS-3 (eNOS) isoform is constitutively expressed by the endothelium and the NO produced by this enzyme is responsible for the relaxation of vascular smooth muscle. NO can diffuse to subjacent smooth muscle cells, where it activates soluble guanylate cyclase and converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). The activation of cGMP-dependent protein kinases leads to the vasodilatation. NO also inhibits leukocyte and platelet activation and adhesion. Vascular endothelium does not only serve as a passive barrier between flowing blood and the vascular wall but also use its strategic location to maintain vascular homeostasis (23). Therefore, loss of endothelial function leads to cardiovascular disease.

3.2.6 Nitric oxide and cadmium

NO is an essential factor in the regulation of the vascular tone and then its effects on blood pressure. Several studies in cell culture and animal models reported that cadmium altered the endothelium function and reduced nitric oxide production (24). Cadmium can also induce the hypertension in rat and mice. Potential mechanisms of cadmium effects on the nitric oxide level include an inhibition of eNOS phosphorylation (9) and expression (8). Interestingly, NO has been reported to protect liver, pituitary and glioma cells from cadmium toxicity. This phenomenon may be explained by a reduction of oxidative stress elicited by cadmium because cytotoxic

ROS can be converted to peroxynitrite via the enzymatic reaction with NO and peroxynitrite seems to play a minor role in cadmium toxicity (17).

3.3 Cadmium

3.3.1 Introduction

Cadmium is a toxic heavy metal, which cannot be degraded by our bodies. It is categorized on the periodic table as a transition element in the same group as zinc and mercury. The atomic number and molecular weight of cadmium are 48 and 112 gram per mole, respectively. The heavy metals are normally generated from human activities such as smelting and refining of lead and batteries industry. As a result, the concentration of heavy metals in the environment increases steadily (25). The International Agency for Research on Cancer (IARC), a part of the World Health Organization, classified cadmium and Cd-containing compounds as group-I human carcinogens based on data obtained from the human occupational exposure in 1993 and animal models (26).

3.3.2 Sources of cadmium

Cadmium is normally produced as a by-product from smelting and refining processes of lead and zinc. Nowadays, cadmium is used in the production of pesticide, pigments and batteries. Mostly, it is found in the compound forms such as cadmium sulfate ($CdSO_4$), cadmium nitrate ($CdNO_3$) and cadmium chloride ($CdCl_2$), which are colorless and soluble in water. Other major sources of cadmium in the environment are the contaminated soil, water, vegetables root, rice, wheat, peanuts and tobacco, which could lead to an accumulation in the food chain (27).

3.3.3 Cadmium exposure

Occupational workers are exposed to cadmium at their workplace by inhalation or skin contact (28). However, the common cause of exposure for general population is the ingestion of contaminated food and cigarette smoking including the

second-hand smoking (25). One cigarette contains approximately 1 to 2 μg of cadmium (29). About 10% of the cadmium content in a cigarette is inhaled during smoking. Normally, cadmium levels in a non-occupational individuals or nonsmokers are usually less than 1 μg/L (30). Cadmium exposure results in a variety of adverse effects in human and animals depending on the dose, route and duration of exposure. Cadmium can damage various organs including the lungs, liver, kidneys, bone, testes and placenta (5). In Thailand, cadmium is highly contaminated in Mae Sot District, Tak Province, during the surveys in 2001-2004 (7).

3.3.4 Toxicokinetics of cadmium

Cadmium is absorbed through the human body by three major routes: ingestion, inhalation and skin absorption. When human consumes a contaminated food or water, 5 to 10% of contaminated cadmium could be absorbed by the gastrointestinal tract (GI). The absorption of cadmium after inhalation ranges from 5 to 35% depending on the specific compounds such as fumes or dusts, site of deposition, and particle size. These factors can influence the penetration of cadmium though different parts of the respiratory system.

Once absorbed, cadmium is transported in blood circulation by binding to albumin or low molecular weight proteins such as metallothionein (MT). Cadmium is rapidly taken up by tissues and primarily deposited in the liver. During the cadmium exposure, the liver synthesizes metallothionein (MT), a metal-binding protein, to bind and detoxify cadmium. A Cd-MT complex is released from the liver after hepatocyte death, and transported through the blood circulation into other organs such as kidneys and bone.

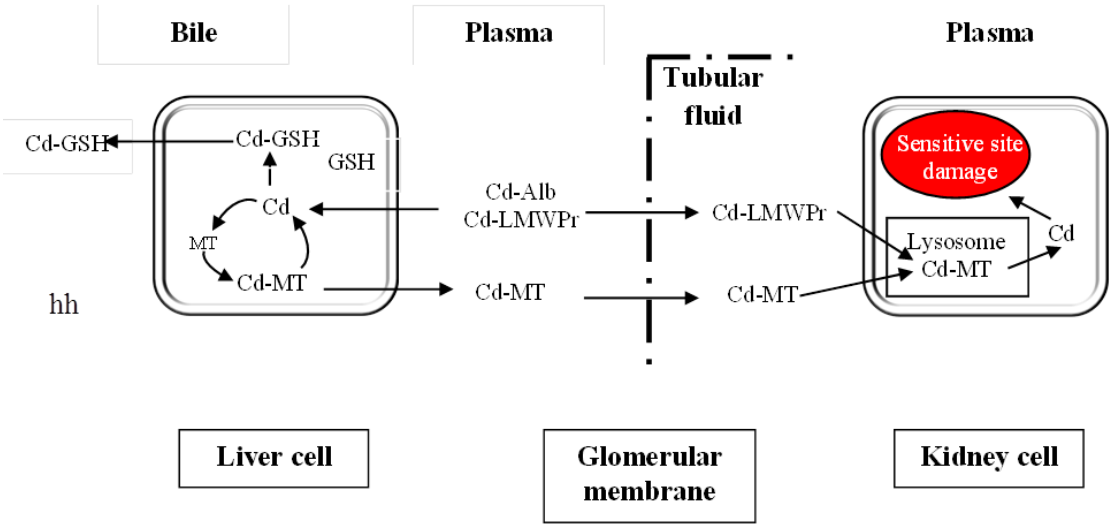

Figure 3.4 Cadmium transportation, protein binding and toxicity (30). Cadmium enters into blood circulation and binds to albumin (Alb), metallothionein (MT) or low molecular weight protein (LMWPr). Cadmium can uptake into liver and forms complex with glutathione (GSH). Cadmium can release from liver and transport to kidney. At the kidney, cadmium complex with MT, Alb or LMWPr filtrate by glomerulus and fuse with lysosome which dissociate cadmium complex. Free cadmium cause the sensitive site damage to renal resulting in renal dysfunction. (GSH, glutathione; MT, metallothionein; Alb, albumin; LMWPr, low molecular weight proteins). Adapted from reference (30).

Cadmium is very poorly excreted. In each day, our body excretes only approximately 0.001% of cadmium. Generally, liver and kidneys are the major organs that show cadmium toxicities. Approximately 50-75% of people receiving cadmium show signs of toxicities. Cadmium has a long biological half-life ranging from 10 to 30 years. A Cd-MT complex is rapidly filtered through the glomerulus. Afterward, cadmium is reabsorbed and fused with lysosome in renal tubular cells, leading to the dissociation of a Cd-MT complex. The generation of free cadmium in renal tubular cells causes cell damage, resulting in the renal toxicity. The relationship between cadmium metabolism and its toxicity is shown in Figure 3.4

3.3.5 Cadmium toxicity

Renal effects

Kidneys are well recognized as target organs for the chronic cadmium toxicity. Renal damage induced by cadmium is a result of proximal tubular reabsorptive dysfunction. The urinary biomarkers of the tubular damage are the low molecular weight proteins found in plasma such as β_2-microglobulin (β_2-M), α_1-microglobulin (α_1-M) and retinol binding proteins. In addition, the lysosomal enzyme N-acetyl glucosaminidase (NAG), a marker of cytolysis, also increases in cadmium exposure (31). Under normal condition, these proteins are filtrated at the glomerulus and reabsorbed efficiently by the renal proximal tubule; therefore, these proteins are not excreted in the urine (28). In case of a renal tubular damage, it has been suggested that the "mild" or "slight" tubular proteinuria (β_2-microglobulin in urine between 300 and 1000 μg/g creatinine) might be reversible (31). However, a urinary level of 1,000 μg of β_2-microglobulin per gram of creatinine is an indicator of the irreversibility of renal toxicities (28).

Bone effects

It has been known since 1950's that chronic cadmium exposure can cause a bone disease known as "itai-itai", whose symptoms are osteomalacia, osteoporosis and kidney failure. It has been suggested that the 2-4 μg of urine cadmium per gram of creatinine or chronic cadmium exposure increased the risk of bone fracture (31). Jarup and colleagues hypothesized that Cd may disrupt the bone metabolism by direct and indirect mechanisms. Direct toxicity implies that the low nephrotoxic Cd levels in blood reach to the bone compartment and exert its toxicity on bone cells. In contrast, circulating cadmium causes the damage of renal proximal tubular cells, leading to the decrease of vitamin D_3 activation, the reduction of intestinal calcium absorption and the subsequent bone remodeling (Figure 3.5) (32).

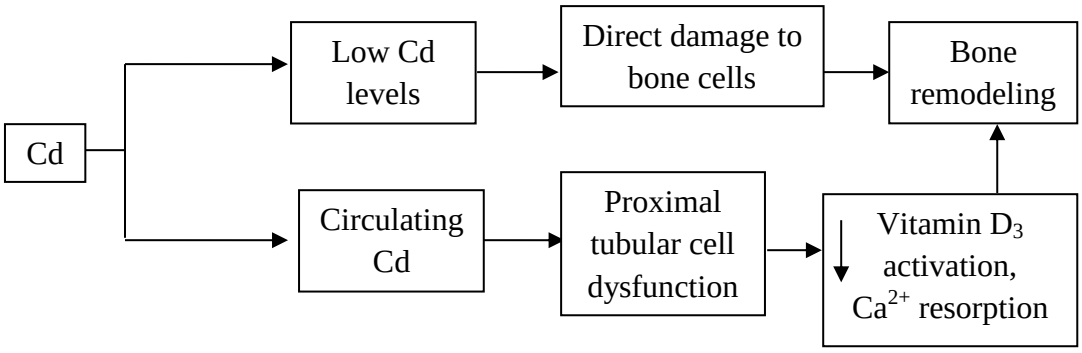

Figure 3.5 The hypothetical model of cadmium effects on bone cells leading to the bone remodeling

Cancers

In 1993, cadmium is recognized as a category I human carcinogen by the International Agency for Research on Cancer (IARC) based on evidences of carcinogenicity found in both human and experimental animal models (26). It is first identified as a weak mutagen and poor initiator of cancers by measuring its interaction with DNA. However, latter several studies reported that cadmium acts through the epigenetic pathway or other mechanisms including mitogenic effects on gene expression, DNA repair inhibition, and inhibition of apoptosis (33). It should be noted that acute cadmium toxicity could induce the alteration of DNA methylation (Figure 3.6) and the long-term exposure at low dose could result in the hypermethylation of DNA. A previous study in transformed prostate cells reported that cadmium induced DNA hypermethylation by increasing the activity of DNA methyltransferase. The reduction of the tumor suppressor gene expression mediated by the Cd-induced DNA hypermethylation could play a role in Cd-induced malignant transformation in human prostate cells (34).

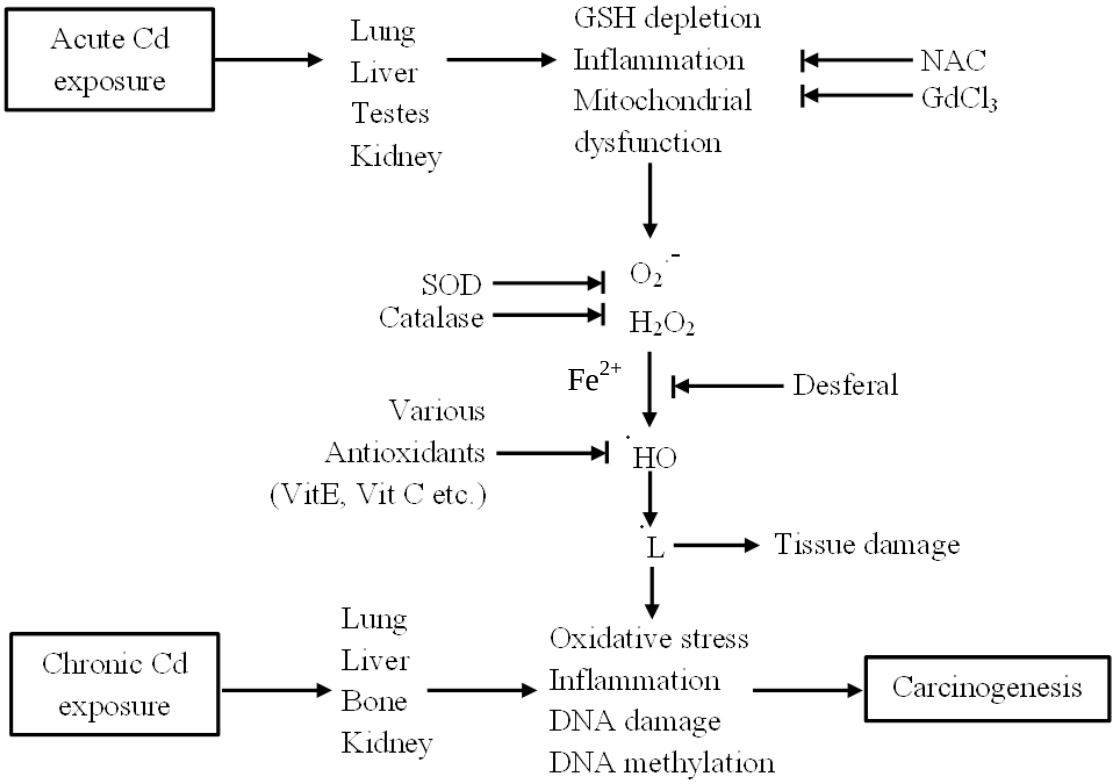

Figure 3.6 Proposed pathways for reactive oxygen species generation and oxidative stress in cadmium toxicology and carcinogenesis following acute or chronic exposure (34)

Reactive Oxygen Species (ROS)

Several studies reported that cadmium could cause oxidative stress leading to the endothelial dysfunction and increased endothelial permeability. However, cadmium concentration at 1-2 µM induced the increases of glutathione, thiol-enzyme activities with minimal change in barrier function and ATP content (35). It has been suggested that the mechanisms of acute cadmium toxicity are the depletion of glutathione and protein-bound sulfhydryl group leading to the increased production of ROS such as superoxide ion, hydrogen peroxide, and hydroxyl radical (36). The redox reaction cannot be generated by cadmium in the biological systems. In contrast, cadmium has been shown to induce the ROS production via an indirect mechanism by damaging critical organelles such as mitochondria.

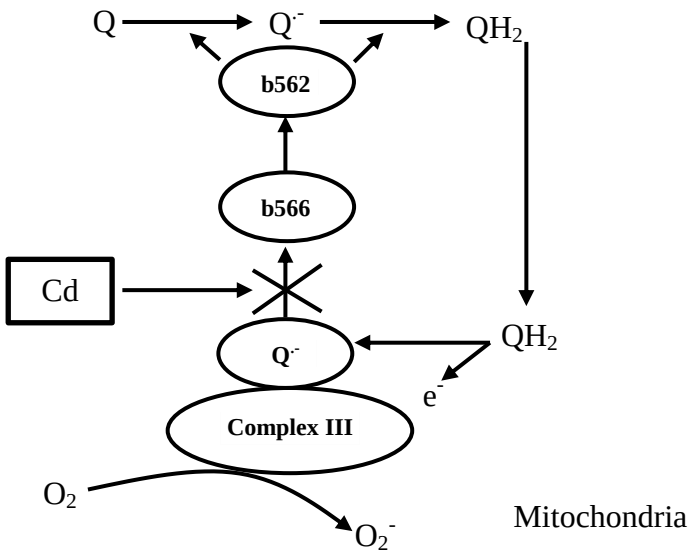

Figure 3.7 Cadmium interrupts the electron transport chain in mitochondria (37)

The inhibition of the complex III of electron transport chain results in the accumulation of semiubiquinones at Q0 site, which are unstable. The electron transfer from semiubiquinones to molecular oxygen creates hydrogen peroxide (H_2O_2) (Figure 3.7). In addition, the ROS formation may deplete the endogenous intracellular radical scavengers such as glutathione or protein sulfhydryl (e.g. thioredoxin) (17).

Vascular Effects

The vascular endothelium plays a major role in regulating vessel tone, homeostasis, and anti-thrombosis. It serves as a selective barrier between the blood stream and the vessel wall. One of the proposed mechanisms of Cd-induced cardiovascular diseases (CVDs) are the impairment of vascular endothelial functions and the damage of primary endothelial layer. The disruption of endothelial cell-cell adhesions by cadmium exposure results in the loss of barrier function and cell death, leading to the initiation of atherosclerosis - the basis of most CVDs (6). Cd also promotes the proliferation of vascular smooth muscle cells and enhances the production of extracellular matrix components that increase the stiffness of blood vessels. In addition, cadmium inhibits the release of endogenous vasodilator such as PGI_2 and NO (5).

Cadmium and vascular endothelial cells

Several studies reported that cadmium induced vascular diseases such as atherosclerosis. Therefore, the vascular endothelium may be an important target of cadmium toxicity. Cadmium exposure reduces the endothelial barrier function by disrupting endothelial cell-cell adhesions and causing endothelial cell death based on *in vivo* and *in vitro* studies (6). The previous study reported that cadmium could induce alterations in the endothelial integrity of other tissues including lung, uterus, nervous system, placenta and liver (5). However, at the non-lethal level, 10 μM, cadmium enhanced the expression of metallothionine in human coronary artery endothelial cells (HCAECs) which was responsible for detoxifying cadmium toxicity (38). The previous study also reported that cadmium and mercury ions caused endothelial dysfunction in bovine pulmonary artery endothelial cells. After 48 hour of exposure, heavy metal ions at concentrations greater than 3–5 μM, produced profound cytotoxicity by increasing lactate dehydrogenase leakage, causing a permeability barrier failure, as well as depleting glutathione and ATP. The low level of glutathione and ATP seem to be the result of a complete inhibition of the activities of key thiol enzymes including glucose-6-phosphate dehydrogenase (G6PDH) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). However, the concentrations of heavy metal less than 1–2 μM induced the increase of glutathione and thiol-enzyme activities with minimal changes in LDH leakage, barrier function and ATP content (35). In addition, it has been demonstrated that Cd induced matrix metalloproteinase (MMP)-2 and MMP-9 activation in a pulmonary emphysema rat model. Both MMP enzymes have been shown to be present and active in atherosclerotic plaques and are responsible for the degradation of type IV collagen. An *in vivo* study showed that MMPs might further enhance the disruption of endothelial cell integrity and might promote the plaque vulnerability (39). A recent study reported that Cd at low concentration promoted the autophagy and depressed the levels of integrin β4, caveolin-1 and PC-PLC activity in human vascular endothelial cells (HUVECs) (40). Transportation of Cd through endothelial cells is involved with several ion channels and transporters.

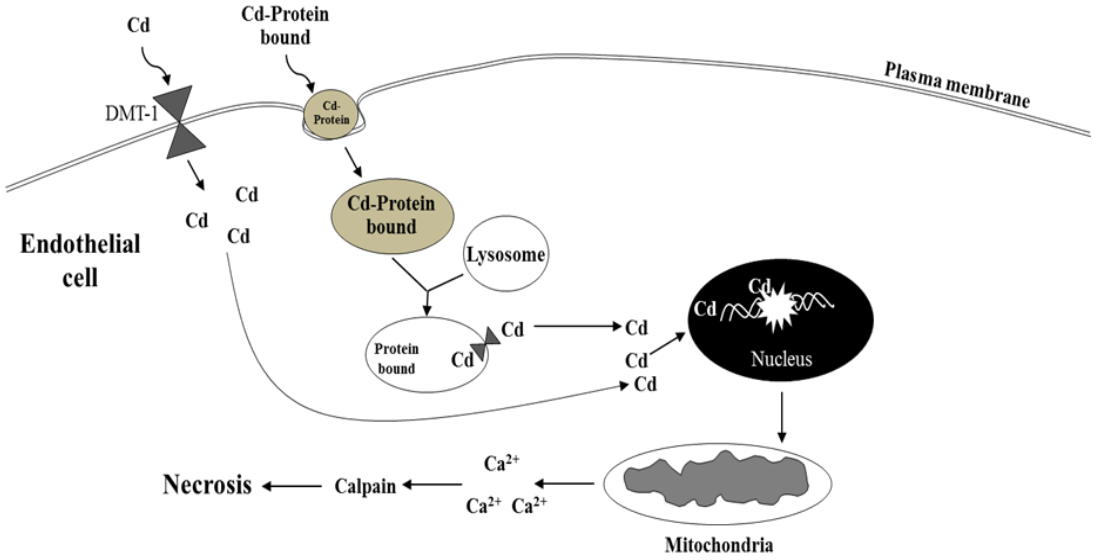

Figure 3.8 A hypothetical model of cadmium-induced necrotic endothelial cell death. Cadmium is taken up by endothelial cells either directly via membrane associated ion transporters and ion channels e.g. divalent metal transporter 1 (DMT-1) or in a protein-bound form via an endocytosis pathway. After cadmium-protein complex enters endothelial cell, the complex fuses with lysosomal membrane leading to dissociation of cadmium from protein. Then, free cadmium diffuses into nucleus and induces DNA damage and a cell death signal sends to mitochondria. Elevation of calcium and activates calpain releasing and induces necrotic cell death**.** Adapted from (6).

Especially, endothelial cells are not a uniform cell type but consist of several cell types, depending on the type of vessels (artery, vein or lymphatic system), vessel diameters, flow conditions, and the surrounding tissues. Cadmium can be taken up to the cells in both free and bound forms. In physiologic condition, the amount of free cadmium in the circulation is very low compared to bound cadmium. Therefore, the main pathway of cadmium to enter cells is the endocytosis of protein-bound cadmium. After that, endosomes fuse with lysosomes and release the free cadmium at the low pH. Ionic cadmium is transported into the cells via the divalent metal transporter 1 (DMT1) as shown in Figure 3.8. Importantly, it has been proposed that the situation (bound to free ion) in the circulation may not reflect the situation in the microenvironment on the surface of endothelial cells. Another route for cadmium to

enter into the endothelial cells is through ZIP8, which is mainly responsible for free cadmium uptake of testicular endothelial cells.

Cadmium and atherosclerosis

Atherosclerosis is the pathophysiology of vascular disease. The initiated step of disease occurs from function and morphology changing in the vascular endothelium lead to increase the permeability of lipid passes through the endothelium and enhance the infiltration of vessel wall by macrophages. Due to LDL easily oxidize and take up by macrophages in uncontrolled way and differentiate into a foam cells. Foam cells deposit in sub-endothelial space, called “fatty streaks”. In addition, the lipid overload by macrophage causing necrotic cell death which stimulate chronic inflammatory occurrence. These conditions stimulate the remodeling process by initiation of cell proliferation and migration lead to increase the thickening intima and medial layer of the vessel wall. All these occurrences are contributed to formation of atherosclerotic plaque. The effects of atherosclerotic plaque formation are allowed such as hypoxia and organs downstream occlusion can be developed to Coronary Heart Disease (CHD). Cadmium associate with atherosclerosis by entering into the endothelial cell, sub-endothelial, and taking up by smooth muscle cell which stimulate cell proliferation lead to thickening of the medial layer of vessel wall via divalent metal transporter-1 (DMT-1) (Figure 3.9A). Also, cadmium disrupts cell-cell junction bond and enhance facilitating the lipids and the immune cells access into medial layer of the vessel wall (Figure 3.9B and C). The uptake overloads of oxidized LDL by macrophages (Figure 3.9D), and then macrophage rupture the foam cells promoting the proliferation chemotactic cytokines as a response to stress (Figure 3.9E). Cadmium further promotes an inflammatory state in the neighboring vessel wall (Figure 3.9F) and initiates the atherosclerotic process (6).

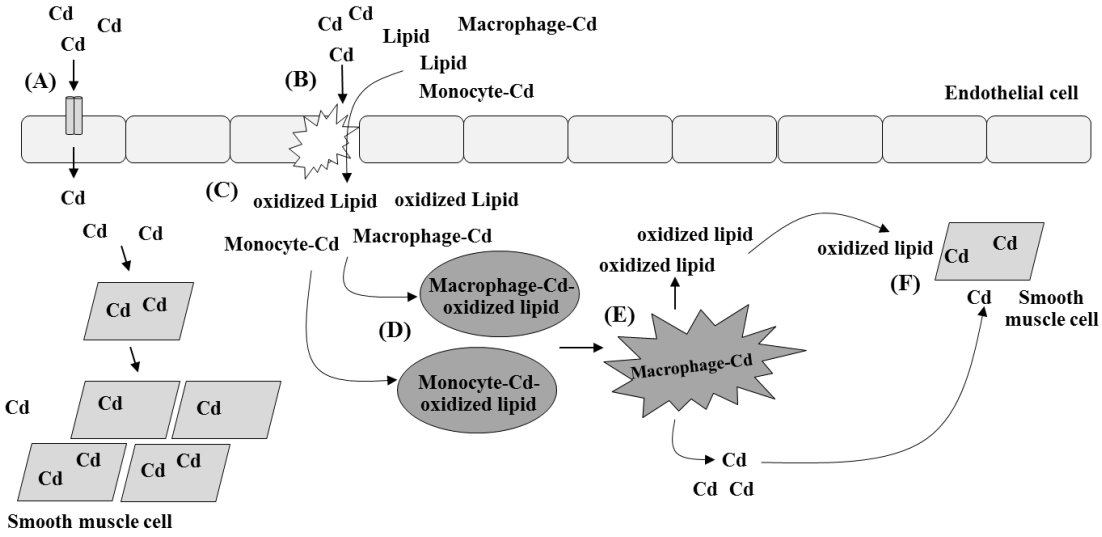

Figure 3.9 Cadmium induces atherosclerosis in the vascular wall. Cadmium may induce and promotes arthrosclerosis. Cadmium is uptake into endothelial cell by divalent metal transporter-1 (DMT-1) (A). Cadmium allows to sub-endothelial cell, and smooth muscle cell, activates smooth muscle cell proliferation lead to thickening of vessel wall. Cadmium disrupts cell-cell junction, not only facilitate cadmium into endothelial cell but also immune cell and lipids (B). Excessive of lipids, they are oxidized by oxidative stress (C), uptake by immune cells such as macrophage and monocyte (D). Immune cells take up oxidized lipid overloads, then rupture and release of cadmium as well as oxidized lipid lead to foam cell formation (E). Cadmium and oxidized lipid transport into neighboring cells (F) which initiates step of atherosclerosis progression. Adapted from (6).