CHAPTER II

LITERATURE REVIEWS

1. ATHEROSCLEROSIS

Atherosclerosis is a disease of the arterial wall characterized by the accumulation of cholesterol deposits in macrophage in large- and medium- sized arteries (Keaney, 2000). The lipid-enriched macrophages called foam cells increase instantly inside arterial wall which reduce the lumen of artery and impede blood flow to terminal tissue particularly heart tissue. Moreover, plaque instability leads to plaque disruption which triggers platelet aggregation, blood clotting and severe obstruction of the artery contributing to ischemic heart disease and heart failure. Atherosclerosis is considered to be the major cause of mortality in western population and elsewhere in the world resulted from many factors. First of all, the progression of atherosclerosis inconspicuously occurs in adolescent and young adults, develops gradually and discovers in middle age of life when the atherosclerotic plaque is detected (McGell et al., 2000). Secondly, high quality of health-care leads to declining of infectious diseases as well as increasing of population lifespan, in addition to expanding of chronic diseases. Thirdly, at present, population around the world confronts various risk factors of atherosclerosis such as obesity, cigarette smoking, hypertension, diabetes and abnormal lipids (Vinereanu, 2006).

The progression of atherosclerosis originates from the endothelial dysfunction caused by free radicals, infectious microorganisms, shear stress, hypertension, toxins after smoking (Singh et al., 2002). Impaired endothelial cell secretes nitric oxide, a volatile gas which causes smooth muscle relaxation and vasodilatation. Apart from vasodilatation effect, nitric oxide also reduces adhesion molecules synthesis such as VCAM-1 and ICAM-1 (Qian et al., 1999) which supports leukocyte to infiltrate into intimal layer of artery. Furthermore, nitric oxide reduces platelet aggregation, reduces pro-inflammatory cytokines expression and inhibition of nuclear factor kappa B (NF-κB) signaling pathway. Therefore, overall consequences lead to infiltrate of leukocyte into vessel and inflammation (Esper et al., 2006).

Afterward, LDL is modified in vascular wall by lipid oxidation, resulting in the formation of so-called minimally oxidized LDL (mmLDL). This molecule is

detected as unself molecule and triggers the inflammatory response. Coincidentally, resulted from explosion of oxidative stress condition, mmLDL is progressively transformed to be oxLDL. Oxidized LDL and its component, oxidized phospholipids and short-chain aldehydes, positively return to elicit the expression of adhesion molecules especially VCAM-1 on vascular endothelial cells and also stimulate the expression of chemoattractant molecules including monocyte chemoattractant protein (MCP)-1. As results of adhesion molecules and chemoattractant molecule expression, leukocyte repetitively adheres and penetrates endothelial layer into the arterial intima and provokes inflammatory mediators including pro-inflammatory cytokines and small lipid molecules such as leukotrienes and prostaglandins. Moreover, these components can generate reactive oxygen species which increase oxidative status (Woo et al., 2002).

Macrophage engulfed oxLDL transforms itself to foam cell. It contains various kinds of lipid oxidation products which are potent mediators of atherosclerosis. In particular, the importance of foam cell formation is that foam cell accumulation areas are prone to rupture by subsequent activities of immunological cells in artery (Jerome, 2006). Therefore, cholesterol accumulated in foam cell is recognized as an important step in atherosclerosis and therapeutic target. This step is regulated by various genes such as ATP-binding cassette (ABC) proteins and SRs. ABCA1, an apparent transmembrane transporter ABC protein recognized by apolipoprotein (Apo) AI on HDL, has a role in cholesterol efflux in macrophage (Attie, 2007). In the other hand, SR-A and CD36, well-known transmembrane glycoprotein SRs in atherosclerosis, have specific interaction with oxLDL leading to the uptake of oxLDL. Particularly, there is positive feedback mechanism to increase lipid accumulation in macrophage by auto-induction of oxLDL to increase the expression of CD36 which in turn to increase lipid accumulation in macrophage foam cell (Silverstein, 2009). Consequently, in progression of atherosclerosis, macrophage predisposes to increase cholesterol efflux into macrophage itself and initiates cytotoxic effect including triggering apoptosis pathways and disruption of membrane domains (Attie, 2007).

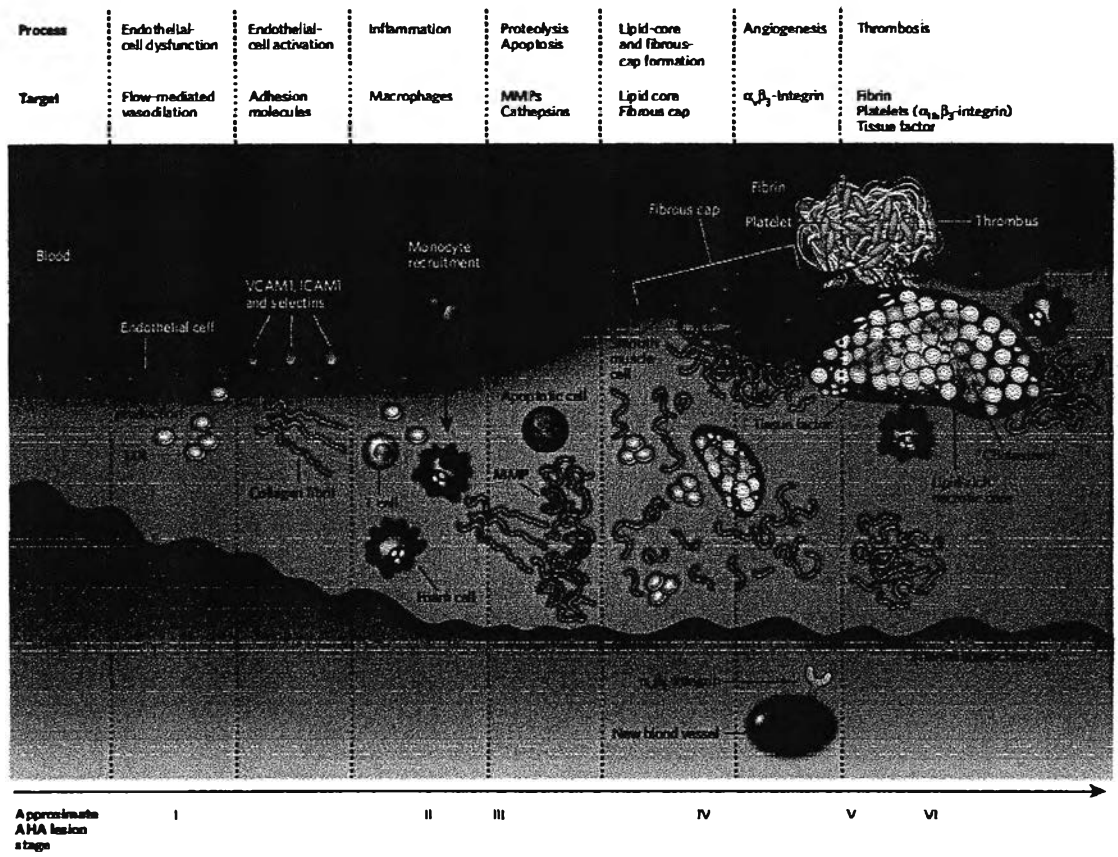

Figure 1 The development of an atherosclerotic lesion (Sanz and Fayad, 2008).

In addition of involving in cholesterol efflux from macrophage, HDL also takes an anti-inflammatory role in atherosclerosis since it contains paraoxonase-1 enzyme which can inactivate pro-inflammatory phospholipids. In addition, HDL also decreases the expression of adhesion molecules on endothelial cell. Therefore, reduction of HDL leads to deprivation of endogenous antioxidant and anti-inflammatory particle (Barter et al., 2004; Navab et al., 2007).

In addition to macrophage accumulation in artery, mast cell or T-cell continuously migrate from vessel lumen result in inflammatory response in artery. Coincidentally, smooth muscle cell resided quiescently in media layer is activated, relocates to intimal layer, and voluntarily motivates extracellular matrix molecules synthesis, including collagens, elastin, and glycosaminoglycans, contributing to fibrous cap stability. However, the progressive inflammation induces the expression of the extracellular matrix destruction enzyme including matrix MMP, cathepsin. Both enzymes and some cytokines including IFN-γ involve in the progression of atherosclerosis and plaque destabilization. Eventually, the rupture from plaque

destabilization stimulates cytokine inducing platelet aggregation and blood clotting. Obstacle of blood flow impeding the oxygen to supply to coronary artery, ischemia occurs resulting in myocardial infarction (Adams et al., 2000).

2. CYTOKINES INVOLVED IN ATHEROSCLEROSIS

The communication of cells in immunological system achieves by using the protein cell regulators, called cytokines. This term was defined by Balkwill and Burke (1989) as a group of protein cell regulators including lymphokines, monokines, IL, IFN and chemokines. Cytokines are produced by a wide variety of cells and play an important role in many physiological responses, involved in the pathophysiology of range of diseases and have therapeutic potential (Tedgui and Mallat, 2006). Subfamilies of cytokines are divided into two sides of effects, pro- and anti-inflammatory effects. Pro-inflammatory cytokines including IL-1β and TNF-α are among the most important cytokine mediators of the inflammatory response. This group of cytokines is mainly secreted by T_H1 and macrophage. Besides IL-1β and TNF-α, IL-12, IL-18, and IFN-γ are also determined as pro-inflammatory cytokines. In contrast, IL-4, IL-10, IL-13, transforming growth factor (TGF)-β are considered to be anti-inflammatory cytokines. Some cytokines such as IL-4, IL-6, GM-CSF are characterized as both pro-inflammatory and anti-inflammatory cytokines (Kleemann, Zadelaar, and Kooistra, 2008) (Figure 2).

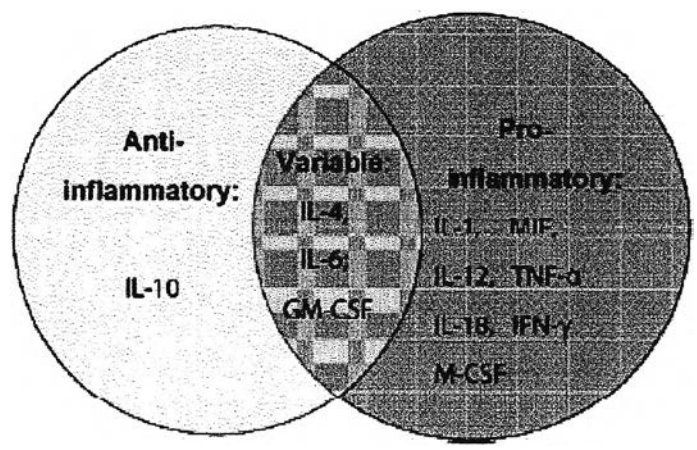

Figure 2 Categorization of cytokines into pro-atherogenic and anti-atherogenic cytokines (Kleemann et al., 2008).

INTERLEUKIN-1β

IL-1β is cytokine in a group of key pro-inflammatory mediators in IL-1 family which involves in atherosclerosis. The members in IL-1 family cytokines encompass IL-1α (IL-1F1), IL-1β (IL-1F2), IL-1RA (IL-1F3), and IL-18 (IL-1F4). Generally, IL-1β is widely studied than IL-1α although both cytokines are supposed to be indifferent in binding and activating the same receptor as well as effects on target cells (Luheshi, Rothwell, and Brough, 2009). IL-1β is released from vascular smooth muscle cell and endothelium. IL-1β mediated loss of endothelium-dependent relaxation (Chamberlain et al., 2009) increased ICAM-1 and VCAM-1 gene expression in vascular smooth muscle cell which enhanced leukocyte adhesion to vascular smooth muscle cell (Wang et al., 1995). Consequently, vascular smooth muscles in the media layer dislocated and proliferated in intimal layer. In contrast to vascular smooth muscle cell activation, IL-1β, together with IFN-γ, released from T_H1 promotes vascular smooth muscle apoptosis leading to depletion of extracellular matrix and atherosclerotic plaque disruption (Geng et al., 1996). In pathogenic disease of atherosclerosis in IL-1β knockout mice (Kirii et al., 2003; Chamberlain et al., 2009), the lesion of aorta in ApoE and IL-1β double-deficient ($ApoE^{-/-}$/$IL\text{-}1\beta^{-/-}$) mice was smaller than ApoE deficient mice ($ApoE^{-/-}$). Moreover, VCAM-1 and MCP-1 expression on the endothelial cell in $ApoE^{-/-}$/$IL\text{-}1\beta^{-/-}$ mice were decreased compared to $ApoE^{-/-}$ mice (Kirii et al., 2003).

MONOCYTE CHEMOTATIC PROTEIN-1

MCP-1 or Chemokine (C-C motif) ligand 2 (CCL2) is a cytokine belongs to chemokine in CC family. MCP-1 is a major monocyte chemotactic factor highly expressed in various cell types including macrophage in areas of atherosclerotic lesion (Ylä-Herttuala et al., 1991). MCP-1 is also found in vascular smooth muscle cell in media layer (Yu et al., 1992) which is induced to release by modified-LDL in endothelial cells (Cushing et al., 1990) accompanying with a variety of cytokines including IFN-γ (Liebler et al, 1994) and TNF-α (Seino et al, 1995). MCP-1 triggered firm adhesion of monocytes to vascular endothelium (Luscinskas et al., 2000) and also stimulated macrophage infiltration into the arterial wall (Namiki et al., 2002). Besides the chemoattracting properties, there are various deleterious effects of MCP-1 including raise of cytotoxic lymphocyte and natural killer cell (Taub et al., 1996),

modification of the phenotype of vascular smooth muscle cells (Denger et al., 1999) and activation of cytokine secretion in monocytes (Reape and Groot, 1999). The importance of MCP-1 in atherosclerotic lesion has been demonstrated by using mouse model characterized by deletion of MCP-1 gene. The MCP-$1^{-/-}$ mice, revealed severe defects in monocyte recruitment to the sites of inflammation (Lu et al., 1998); however, MCP-$1^{-/-}$ mice had far less lipid deposition in artery than wild-type MCP-1 alleles. Accompanying by gene modification such as deletion of LDL receptor or human ApoB transgene, the modified-gene mice showed higher lipid deposition in aorta. Mice lacking LDL receptor and MCP-1 gene, LDL-$R^{-/-}$/$MCP^{-/-}$ mice showed higher area of atherosclerotic plaque because of lacking of LDL receptor's function to remove cholesterol-rich intermediate density lipoproteins (IDL) and LDL from the blood. In addition, deficiency of LDL receptor extremely developed extensive fatty streaks throughout their aortas (Gu et al., 1998). In the other hand, knockout of MCP-1 gene investigating in LDL-$R^{-/-}$/$MCP^{-/-}$ mice showed lower area of atherosclerotic plaque as compared to LDL-$R^{-/-}$ mice. Further study in hypercholesterolemic transgenic mice expressing human ApoB with MCP-1 gene deletion (MCP-$1^{-/-}$ $HuBTg^{+/0}$ mice), overproduction of atherogenic lipoprotein- human ApoB, which raised ApoB level in the liver, elevated plasma cholesterol levels and increased susceptibility to atherosclerosis on a high-fat diet. Dramatically protection from macrophage recruitment and atherosclerotic lesion formation were found in MCP-$1^{-/-}$ Human apolipoprotein B transgenic $(HuBTg)^{+/0}$ mice without altering lipoprotein metabolism as compared to MCP-1 expressed ApoB transgenic mice (Gosling et al., 1999). Concordantly with rabbit model, MCP-1 was also strongly expressed in macrophage-rich regions atherosclerotic lesion and may play an important role in recruitment of monocyte/macrophage into developing lesions (Ylä-Herttuala et al., 1991).

TUMOR NECROSIS FACTOR-α

TNF-α is a pro-inflammatory cytokine with a variety of biological activities released from macrophage (Sprague and Khalil, 2009), endothelial cells and smooth muscle cells (Barath et al., 1990) in atherosclerotic plaque. TNF-α induces the expression of adhesion molecule which aid leukocyte attach to endothelial cells (Cavender et al., 1987) and play a role in chemotactic activity (Ming et al., 1987).

TNF-α is a potent stimulation of several MMPs (Rajavashisth et al., 1999) and plasminogen activator inhibitor (Galis et al., 1995). In the study of $ApoE^{-/-}$ mice which demonstrated hypercholesterolemia on a normal chow diet, with a prominent increase in the level of very low density lipoprotein (VLDL) and decrease in the level of HDL, there were no difference in total cholesterol (TC) levels between compound-deficient mice in ApoE and TNF-α ($ApoE^{-/-}$/ TNF-$\alpha^{-/-}$) and $ApoE^{-/-}$ mice. However, the atherosclerotic plaque area in the aortic luminal surface of $ApoE^{-/-}$/ TNF-$\alpha^{-/-}$ mice was smaller than the plaque area in $ApoE^{-/-}$ mice (Brånén et al., 2004; Ohta et al., 2005). Less expression level of ICAM-1, VCAM-1 and MCP-1 was also found in $ApoE^{-/-}$/ TNF-$\alpha^{-/-}$ mice as compared to $ApoE^{-/-}$ mice (Ohta et al., 2005). In patients with unstable angina pectoris, TNF-α concurrently with IL-1β, IL-2 and IL-8 were detected in higher amount as compared to patients with normal coronary arteries (Ozeren et al., 2003). Therefore, overall effects of TNF-α in atherosclerosis were characterized as pro-inflammatory cytokine.

INTERLEUKIN-10

IL-10 or previously known as cytokine synthesis inhibitory factor (CSIF) is a cytokine released from monocyte (de Waal Malefyt et al., 1991) and T_H2 cell (Fiorentino, Bond, and Mosmann, 1989) by various kinds of stimulator including LPS and oxidized lipids (Cushing et al. 1990). IL-10 associates with numerous functions in anti-inflammation. For example, IL-10 inhibited antigen-presentation capacity of macrophages and dendritic cells, blocked cytokine production including IL-1β, IL-6, IL-8, TNF-α, GM-CSF และ G-CSF in LPS-induced monocyte (de Waal Malefyt et al., 1991). These may be resulted from activation of the suppressor of cytokine-signaling (SOCS)-1 gene producing the protein that inhibits pro-inflammatory cytokine through JAK-STAT pathway (Pestka et al. 2004) and NF-κB pathway (Wang et al., 1995). In balance between pro-inflammatory and anti-inflammatory effect in immunological system, IL-10 showed prominent roles in inhibition of T_H1 immune responses which aggravate progression of atherosclerosis such as down-regulation of pro-inflammatory cytokine IL-12 production from T_H1 (Uyemura et al., 1996). Furthermore, IL-10 also inhibits chemokine secretion and modifies chemokine receptor expression (Terkeltaub, 1999). IL-10 not only inhibits cytokine expression and release but also

prevents atherosclerosis plaque degradation from MMP-9 released by IL-18-stimulated or unstimulated peripheral blood mononuclear cell (PBMC) (Nold et al., 2003). The anti-inflammatory cytokine IL-10 also enhances the secretion of tissue inhibitor of metalloproteinases-1 (TIMP-1) which counteracts MMPs effects (Nold et al, 2003). In the study of IL-10 gene using knockout mice model, IL-10-deficient C57BL/6J mice placed on an atherogenic diet for 16 weeks showed an increment of aortic lesion as compared to wild-type mice. Moreover, IL-$10^{-/-}$ mice exhibited the increased T-cell infiltration, IFN-γ expression and decreased collagen content. However, intramuscular injection with plasmid expressed IL-10 to produce IL-10 cytokine into IL-$10^{-/-}$ mice significantly reduced fatty lesion development (Mallat et al., 1999). Therefore, lacking of an anti-inflammatory cytokine IL-10 initiates aggressive lesion development and propagation of atherosclerosis.

TRANSFORMING GROWTH FACTOR-β

TGF-β is one of the cytokines released from a range of cell types in vascular wall including endothelial cell, smooth muscle cell, monocyte, macrophage, platelet, regulatory T cell (T_{reg}) and myofibroblasts. Presently, TGF-β superfamily encompasses 35 members which are found in vertebrates and invertebrate organisms. However, the major types of TGF-β in mammals are TGF-β1, TGF-β2 and TGF-β3. Each of these cytokines is constructed from different genes. However, these cytokines contain about 70 – 80% identical sequence homology. Their properties, at least *in vitro*, were not different. In gene expression, TGF-β synthesis is regulated by a range of factors at the level of gene transcription. Especially, TGF-β1 can auto-induce its mRNA expression by interaction with activator protein-1 to their particular sequence in the promoter region. After TGF-β bind to TGF-β receptors especially TGF-RII, the signaling cascades through classical pathway. The intracellular signaling pathway originates from recruitment of TGF-RI to form a heteromeric complex consisting TGF-RI and TGF-RII. Subsequently, phosphorylation of TGF-RI at multiple serine and threonine residues in the GS domain lead to its conformational change and then the activation of Smads, a novel family of signal transducers, occur. Consequently, phosphorylated Smads translocates into the nucleus and interacts with Smad binding elements (SBEs) in the promoter or enhancer regions of target genes (Derynck and Zhang, 2003). In addition to regulation of gene transcription, signaling from Smads

can also modulate gene transcription via protein-protein interactions. The interactions were studied in various cells in the vascular wall including monocytes, macrophages, vascular smooth muscle cells, endothelial cells. TGF-β was shown to inhibit the effects of pro-inflammatory agent induced the expression of a number of adhesion molecules such as P-selectin, E-selectin and ICAM-1 (DiChiara et al., 2000) which assisted adherence of monocyte to endothelial cell by binding on these adhesion molecules. In macrophage, numerous gene expressions such as MMP-12, iNOS, pro-inflammatory cytokines including MCP-1 induced by LPS were suppressed by TGF-β. Moreover, TGF-β was able to reduce inflammatory responses by inhibition of nitric oxide and superoxide radicals which involved in inflammatory response but increased anti-inflammatory IL-10 expression (Maeda et al., 1995). In cholesterol uptake to and efflux from macrophage, TGF-β attenuated the expression of SR, the cell-surface membrane proteins which internalized modified lipoprotein and then initiated transformation of macrophage to be foam cell (Draude and Lorenz, 2000; Ramji et al., 2006). In the other hand, TGF-β has been found to increased the expression of ABC transporter including ABCA1, ABCG1 that stimulated macrophage cholesterol efflux (Werner et al., 2000; Panousis et al., 2001). TGF-β stimulated the synthesis of proteoglycans and extracellular matrix proteins in vascular smooth muscle (Grainger et al., 1994) and also stimulated the expression of the IL-1RA, an anti-inflammatory cytokine in IL-1 superfamily (Di Febbo et al., 1998). In addition, TGF-β promoted differentiation of smooth muscle cell but inhibited the effects of pro-inflammation cytokines that increased the proliferation and migration of smooth muscle cell. The stable atherosclerotic lesions expressed greater amounts of TGF-β expression than the unstable atherosclerotic lesions (Cipollone et al., 2004). Moreover, overexpression of TGF-β caused lower expression of pro-inflammatory cytokines, MMP-13, and retarded aortic root atherosclerosis (Frutkin et al., 2009). These characteristics of TGF-β indicated its advantageous effects of TGF-β in atherosclerotic lesions.

2. LIVER INFLAMMATION AND INJURY IN AN ASSOCIATION TO ATHEROSCLEROSIS

Liver is a vital organ which has a wide range of function such as nutritious substance catabolism and synthesis as well as xenobiotic biotransformation. Cellular structure of liver composes of parenchymal liver cells (hepatocytes), Kupffer cells, hepatic stellate cells, sinusoidal endothelial cells (Figure 3).

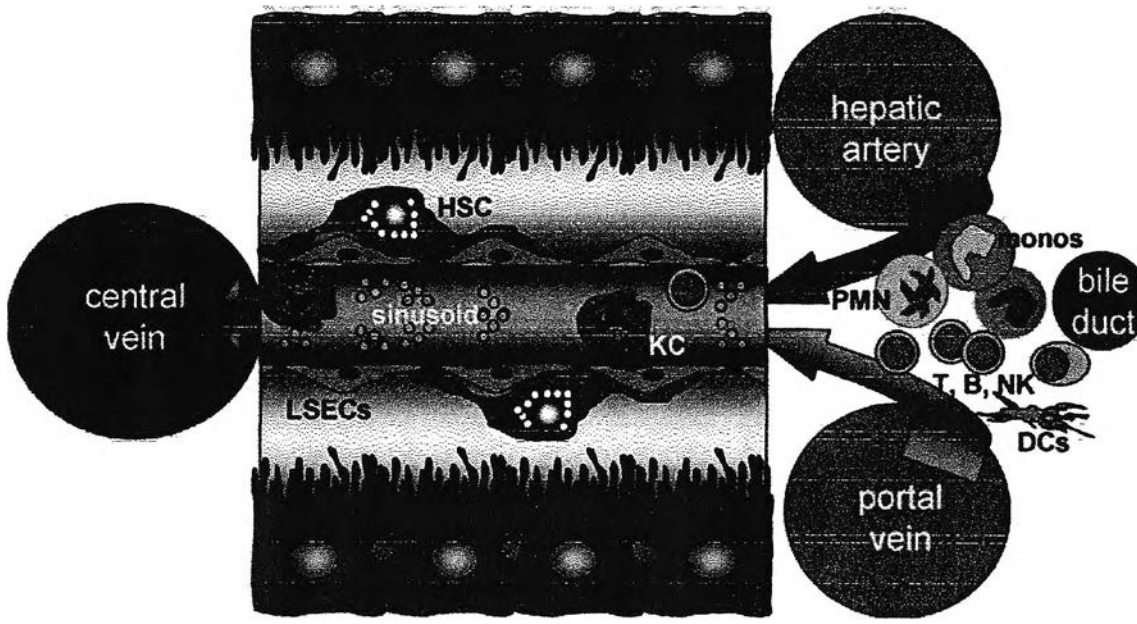

Figure 3 Liver cells showing liver sinusoidal endothelial cells (LSEC); Kupffer cells (KC), hepatic stellate cells (HSC), as well as the inflammatory associated cells: polymorphonuclear neutrophils (PMN), monocytes (monos), dendritic cells (DCs) (Tacke et al., 2009).

Mechanism of liver inflammation and injury

In xenobiotics metabolism, substances received by ingestion mainly pass through liver; therefore, this organ usually contacts to various kinds of xenobiotics and prones to damage. Unexpectedly, hepatocellular damage is not due to direct damage of xenobiotics; however, most cases are from inflammatory cells attracted by the stress of hepatocytes. Initiation of liver inflammation from xenobiotic is occurred when unself molecules that trigger Kupffer cells, macrophages located in liver (Hoebe et al., 2001). These cells are the center of inflammatory response which release wide range of cytokines such as IL-1β, IL-6 and TNF-α as well as chemokine mediated

immigration of leukocytes into the liver (Diehl, 2000). In normal situation, TNF-α released by Kupffer cells is essential for normal liver regeneration and also augment liver DNA synthesis depended on NF-κB activation (Marra, 2009). TNF-α is proposed to assist liver regeneration by priming hepatocytes to respond to TNF-α and growth factors such as hepatocyte growth factor (HGF) and epidermal growth factor (EGF). However, TNF-α is implicated as a prominent pro-inflammation cytokine in liver inflammation and proposed its actions to initiate apoptosis by binding to its receptor, TNF-R1 and TNF-R2. The interaction between cytokine and its receptor mediates the transcription factor NF-κB and triggers caspase activities lead to apoptosis signaling (Bradham et al., 1998). Moreover, Kupffer cells in liver inflammation consequently synthesize inflammatory mediator such as oxygen radicals, eicosanoids, platelet-activating factor, and cytokines including IL-1β, IL-6, and TNF-α (Decker, 1990). The other well-studied cytokine which shows prominent roles in liver inflammation is TGF-β. In normal situation, TGF-β was found to inhibit DNA synthesis in liver regeneration (Michalopoulos, 1990). In contrast to the function of TGF-β in artery, TGF-β expressed by liver obviously exhibits pro-inflammatory effects including cell adhesion, cell migration, and extracellular matrix deposition which contribute to accumulation of extracellular matrix resulting in liver fibrogenesis (Meindl-Beinker and Dooley, 2008).

In acute liver injury (Figure 4), hepatotoxins such as drugs, infectious agents induce a stress situation in hepatocytes that trigger the release of chemokines and coincidentally release pro-inflammatory cytokine such as TNF-α which induces the expression of adhesion molecules. These situations support leukocyte to immigrate to liver and subsequent hepatocellular damage (Ramadori and Armbrust, 2001).

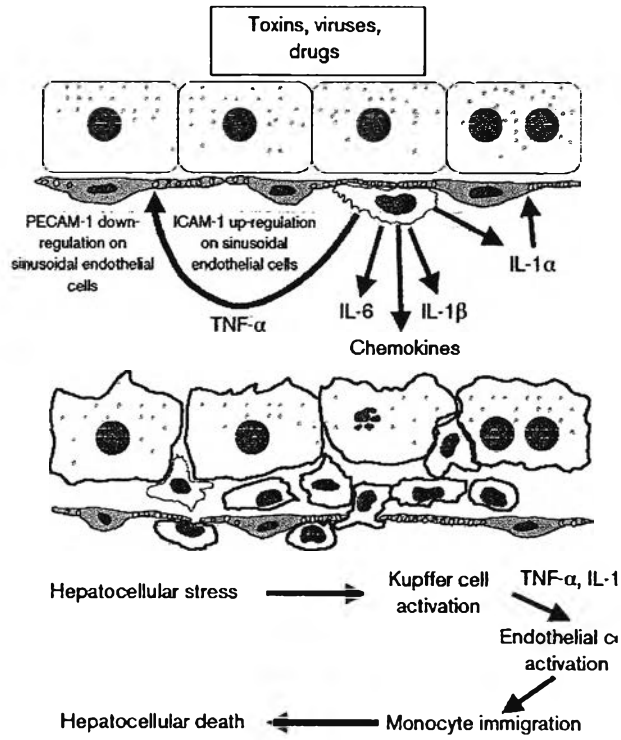

Figure 4 Acute liver injury and the involvement of cytokines (Ramadori and Armbrust, 2001); ICAM-1, intracellular adhesion molecule-1; IL-1, interleukin 1; IL-6, interleukin 6; PECAM1; platelet endothelial cell adhesion molecule-1; TNF-α, tumor necrosis factor alpha.

In chronic liver injury (Figure 5), hepatocyte stellate cells play an important role in perpetual liver damage. Initially, sinusoidal endothelial cells release TGF-β and TNF-α that trigger hepatocyte stellate cell activation. Resulted from the activation, hepatocyte stellate cell tends to release MCP-1 and subsequently recruits mast cells which in turn to trigger positive feedback activation to hepatocyte stellate cell. Accompanying with leukocyte deposition by adhesion molecule expression from sinusoidal endothelial cells, the loop of downright activation from hepatocyte stellate cells to inflammation cell leads to chronic injury of liver. Overall effects of loop activation raise the amount of pro-inflammatory cytokine TGF-β and TNF-α to synthesize extracellular matrix in hepatocyte stellate cells and reduce matrix degradation (Ramadori and Armbrust, 2001).

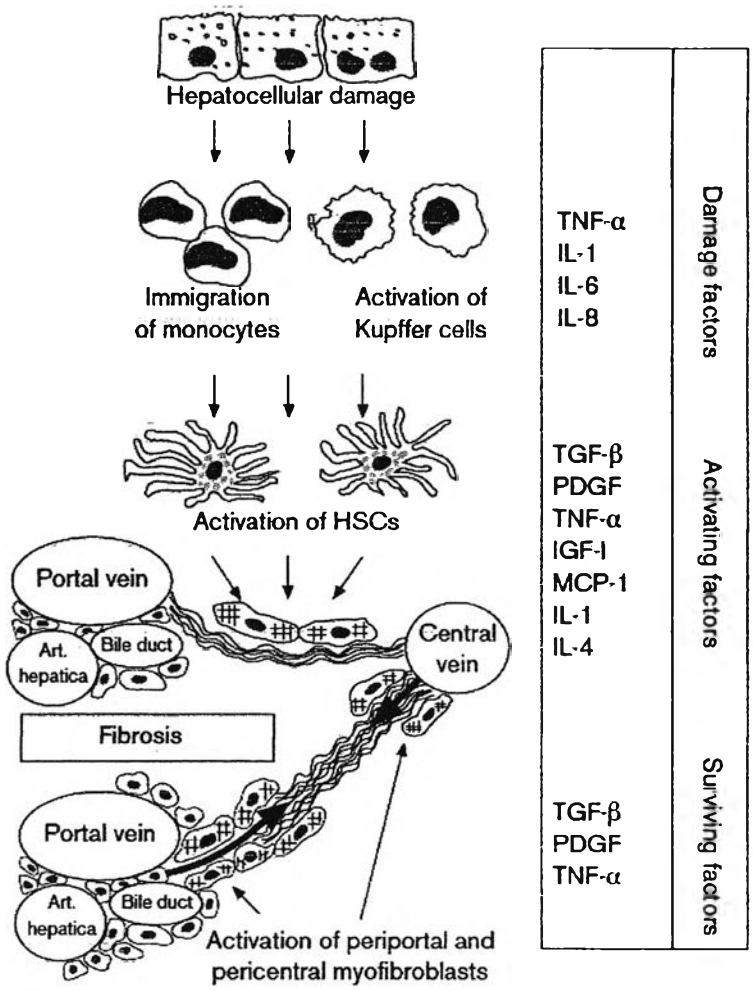

Figure 5 Chronic liver injury and the involvement of cytokines (Ramadori and Armbrust, 2001); IL-1, interleukin 1; IL-4, interleukin 4; IL-6, interleukin 6; IL-8, interleukin 8; MCP-1, monocyte chemoattractant protein-1; PDGF, platelet derived growth factor; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha.

Liver inflammation and injury involved in atherosclerosis

In atherosclerosis study, ApoE knockout mice were used as a typical model with high level of TG as well as development of atherosclerotic plaque in artery which mimic human atherosclerosis. According to Yin et al. (2009), ApoE knockout mice suffered from lipid metabolism dysfunction and inflammation in the liver. That is, expression patterns of cytokines and other pro-inflammatory genes in liver including IL-1β, IFN-γ, IL-6, MCP-1, VCAM-1, and p65 subunit of NF-κB are raised up and plasma cytokines including IL-1β, IFN-γ, and IL-6 are elevated as well (Yin et

al., 2009). Moreover, the relationship between atherosclerosis and liver inflammation was established. ApoE knockout mice fed with high cholesterol diet for 14 weeks exhibited high level of lipid accumulation in adipose tissue, liver and formation of atherosclerotic plaque with macrophage and T-cell infiltrations. Coincidentally, plasma pro-inflammatory cytokine MCP-1 was increased significantly indicated that atherosclerotic ApoE knockout mice fed with high cholesterol diet exhibited widespread inflammation in both atherosclerotic plaque and liver (Lohmann et al., 2009).

There are some relationship between high cholesterol diet, liver inflammation and atherosclerosis. High cholesterol diet is a one of the risk factors of atherosclerosis as well as liver inflammation. In addition, nearly 50% of hyperlipidemic patients have coexisting nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease in Western countries which encompass wide range of liver disease from simple steatosis to nonalcoholic steatohepatitis (NASH). NAFLD is strongly related to an increased cardiovascular risk and atherogenic lipid profile. This study proposed that liver inflammation was associated to development of atherosclerosis and should be one of the risk factors in cardiovascular disease (Alkhouri et al. 2009). In contrast, unclassified chronic liver disease primarily with cirrhosis which studied in post-mortem patients found that hepatic disease patients are less prone to develop complicated coronary atherosclerosis (Otsubo et al., 2006).

3. SIMVASTATIN

The HMG-CoA reductase inhibitors are more commonly known as the statins. Statins were first introduced into clinical practice in the late 1980s after the discovery of their lipid-lowering effect in 1976 when Endo and collaborates isolated the first statin from *Penicillium citrinum*, called mevastatin (Manzoni and Rollini, 2002). Clinical trials of mevastatin quickly discontinued because intestinal tumors were found in dogs (Endo et al., 2004). In general, statins are regarded as a remarkably safe and well-tolerated class of drugs. It must be stressed that on August 9, 2001, cerivastatin was withdrawn from the world pharmaceutical market, after 31 patients died by acute renal failure caused by rhabdomyolysis. As a result, currently, there are seven statins in clinical use: lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, pitavastatin and rosuvastatin (Davidson, 2002).

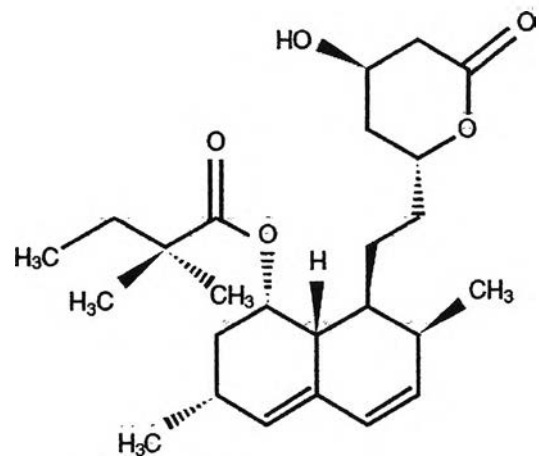

Figure 6 Chemical structure of simvastatin (Schachter, 2005).

Simvastatin, a hypolipidemic semi-synthetic derivative from a fermentation product of *Aspergillus terreus*, was introduced in the late 1980s (Manzoni and Rollini, 2002). The molecular formula of simvastatin is $C_{25}H_{38}O_5$ and its molecular weight is 418.57 (Merck Sharp & Dohme Corp 2010). Its structural formula is shown in Figure 6.

Mechanism of action of statins

Statins are oral systemic agents that suppress cholesterol synthesis by competitive inhibition of the HMG-CoA reductase, the rate-limiting step in the conversion of HMG-CoA to mevalonate, a precursor of cholesterol (Almuti et al., 2006). Inhibition of cholesterol biosynthesis leads to up-regulation of LDL receptors on the hepatocyte cell surface, which results in increased extraction of LDL-C from blood and decreased circulating LDL-C concentration as shown in Figure 7 (Laws et al., 2004). Statins also have beneficial effects on other lipid parameters, including increases in HDL-C concentration and decreases in TG concentration (NCEP-ATP, 2001).

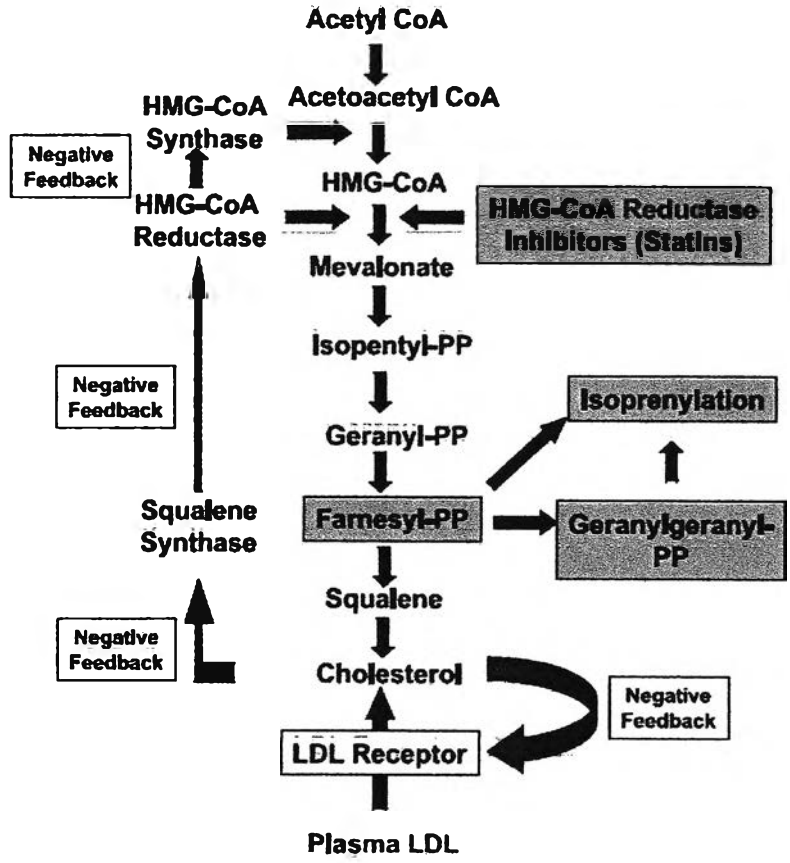

Figure 7 Cholesterol biosynthetic pathway and its inhibition by statins (Laws et al., 2004).

Clinical studies of simvastatin

In 1994, the Scandinavian Simvastatin Survival Study (4S) clearly established that hypolipidemic therapy was safe and reduced morbidity and mortality in hypercholesterolaemic patients with ischemic heart disease (IHD) (Scandinavian Simvastatin Survival Study Group, 1994). Subsequently, Heart protection study (HPS) firmly established the benefit of simvastatin therapy in preventing adverse events in patients at high risk of atheromatous disease, regardless of initial lipid levels (Heart Protection Study Collaborative Group, 2002). In 2004, Phase Z of the A to Z trial demonstrated that acute coronary syndrome (ACS) patients also benefit clinically from high dose simvastatin therapy (De Lemos et al., 2004). However, using simvastatin in clinical practice was found to demonstrate a risk of adverse effects (Grundy et al., 2004).

Anti-atherosclerosis mechanism of simvastatin and other statins

The pleiotropic effects of statins to inhibit HMG-CoA reductase involved in anti-atherosclerotic activity such as inhibition of vascular smooth muscle cells proliferation, prevention of LDL accumulation by macrophages. The results of inhibiting HMG-CoA reductase of statins can be confirmed by the addition of mevalonate that can reverse these effects. In intracellular signaling, statins are proposed to modify cellular signal molecules, including Ras and Rho resulted from reduce mevalonic acid which are important in isoprenoid synthesis. In ApoE knockout mice received fluvastatin 10 mg/kg/day by oral gavage, atherosclerotic plaque disruption was significantly as compared to control ApoE knockout mice. In addition, fluvastatin administration also decreased MMP-9 expression, endothelial adhesion molecules expression, and neutrophil infiltration (Nakamura et al., 2009).

Simvastatin significantly inhibited C-reactive protein- and LPS- induced IL-6 releasing in human monocytes at a concentration of $10^{-8} - 10^{-6}$ mol/l in a dose-dependent manner (Li and Chen, 2003). The expression of IL-6, IL-8 and MCP-1 was significantly decreased in peripheral blood mononuclear cells isolated from hypercholesterolemic patients treated with 20 mg or 40 mg of simvastatin for six weeks (Montecucco et al., 2002). Similarly, besides atorvastatin and cerivastatin, simvastatin also reduced IL-6, IL-8 and MCP-1 in human umbilical vein endothelial cells and peripheral blood mononuclear cells from healthy normolipemic donors (Rezaie-Majd et al., 2002). Simvastatin and atorvastatin suppressed C-reactive protein-induced MCP-1 secretion and ICAM-1 upregulation in human monocytes. They also inhibited chemotaxis by blocking CRP-induced extracellular signal-regulated kinase (ERK) 1/2 phosphorylation, one of the downstream signaling of mevalonate pathway (Montecucco et al., 2009). In clinical study, treatment with 40 mg/day simvastatin for three months significantly reduced MCP-1 but not IL-6 and TNF-α levels in blood of hypercholesterolemic patients without coronary heart disease (Rallidis et al., 2008). Simvastatin and other statins including atorvastatin, fluvastatin and pravastatin reduced IL-6 production in the model of human vascular smooth muscle cells and human mononuclear cells cocultures about 50 – 60 % (Loppnow et al., 2010). Simvastatin also increased serum anti-inflammatory cytokine IL-10 in unstable angina patients treated with 20 or 80 mg of simvastatin in two weeks (Li et al., 2006). In addition to cytokine reduction, simvastatin reduced the

expression of the cytokine involved in thrombosis and plaque instability such as MMP-2, thromboxane A_2 (TXA_2) (Cai and Xie, 2006). In endothelial cells stimulated with phorbol myristate acetate or TNF-α, simvastatin inhibited cyclooxygenase (COX)-2 and MMP-9 expression and activity which triggered plaque inflammation, angiogenesis and plaque rupture. These effects caused by inhibiting mevalonate pathway and subsequent Rho-dependent pathway (Massaro et al., 2009). In overall effect, simvastatin attenuated plaque inflammation (Tahara et al., 2006; Nicholls et al., 2007).

Adverse effects of simvastatin

Simvastatin is generally well tolerated with an adverse effect related withdrawal rate of approximately 3% (Howard-Alpe, Foëx, and Biccard, 2008). The most important adverse effects are liver and muscle toxicities. The definitions of potential muscle adverse experiences due to statins are shown in Table 1. The risk of muscle toxicity with statins therapy may be increased by factors that predispose the patient to myopathy, such as increasing age, female sex, renal insufficiency, hepatic dysfunction, hypothyroidism, diet including grapefruit juice and polypharmacy (Rosenson, 2004; Bays, 2006). The most serious complication adverse effects have been known as rhabdomyolysis and myopathy (Ballantyne et al., 2003).

Effects of statins on liver are found in a small percentage of patients such as elevated liver enzymes, cholelithiasis, cholecystitis, cholestatic jaundice, and liver failure (Kiortsis et al, 2007). Increment of AST and ALT was found in the first 6 months of treatment and discontinuation of statins could reverse elevated liver enzymes. In conventional dose of statins, there were some evidences indicating the elevation of transferase liver enzymes and liver failure occurred in less than 1% and 0.001% of patients, respectively; however, these consequences could not be proved that it was caused by statins. This might be resulted from fluctuations in aminotransferase levels in a population (Cohen, Anania, and Chalasani, 2006). In the other hand, higher dose of statins significantly increase liver transferase enzyme more than three times the upper limit of normal (Armitage, 2007). The cause of liver transferase elevation was also ambiguous due to different conclusions. Tolman KG (2000) proposed that the elevated transferase enzymes of statins treatment arised from decline in LDL cholesterol which was also found in other lipid-lowering drugs such

as bile acid sequestrants, fibric acid derivatives, and nicotinic acid (Tolman, 2000). In contrast, study using univariate regression models to assess for relationship between elevated liver enzymes and reduction of LDL level found that liver transferase level was irrelevant to drop down of LDL but may be relevant to drug- and dose-specific effects of statins itself (Alsheikh-Ali et al., 2007). Recently, treatment with statins was found to cause liver injury by reducing the synthesis, steady-state expression level, and catalytic activity of glutathione peroxidase, that affected selenoprotein biosynthesis (Kromer and Moosmann, 2009).

Table 1 Definitions of potential muscle adverse experiences due to statins (Bays, 2006).

Potential Muscle Adverse Experience	Definitions Used in this Overview
Myalgias	Muscle ache, pain, or weakness with or without CK elevation
Myopathy	Otherwise unexplained elevations in CK $\geq$ 10x the ULN, associated with muscle symptoms (myalgias)
Rhabdomyolysis	Marked CK elevation, typically substantially > 10x the ULN and with creatinine elevation (usually with brown urine and urinary myoglobin). Elevations in other muscle enzymes may also occur, as well as the following: hyperkalemia, hypocalcemia, hyperphosphatemia, hyperuricemia, metabolic acidosis, renal failure, death, and symptoms of muscle weakness may be present, but perhaps only 50% of the time.

CK = creatine kinase; ULN = upper limit of normal

4. *Curcuma comosa* Roxb.

C. comosa Roxb. (Figure 8) is a plant in family Zingiberaceae. It is an indigenous plant of Thailand with a common name in Thai as Waan Chak Mod Look (Tem Smitinand, 2001). Rhizomes of *C. comosa* have been used extensively in Thai traditional medicine as an anti-inflammatory agent particularly for the treatment of postpartum uterine bleeding, peri-menopausal bleeding and uterine inflammation. A number of different active principles of *C. comosa* (Apichart Suksamrarn et al., 1994, 1997) are

1. Diarylheptanoids: *trans*-1,7-diphenyl-5-hydroxy-1-heptene, *trans*-1,7-dephenyl-6-hepten-3-one-5-ol, *trans*-1,7-diphenyl-3-acetoxy-6-heptene, *trans*-1,7-diphenyl-6-heptene-3-one, *trans,trans*-1,7-diphenyl-1,3-heptadien-5-ol, *trans,trans*-1,7-diphenyl-4,6-heptadien-3-one, 1,7-diphenyl-1(*1E,3E,5E*)-heptatriene, 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(*1E*)-1-heptene), 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(*1E*)-1-heptene.
2. Acetophenones: 4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl acetophenone).

Figure 8 *C. comosa* Roxb. plant (A) and rhizomes (B).

Pharmacological effects

Pharmacological effects related to cardiovascular disease and potential toxicities of *C. comosa* have been reported as following:

1. Choleretic effect

Choleretic effect of *C. comosa* had been investigated in rats. The ethyl acetate extract of *C. comosa* showed the greatest stimulatory effects on bile flow rate in rats.

Intra-duodenal administration of the extract caused a dose-dependent stimulation of bile secretion. A high dose (1000 mg/kg) of the extract increased bile flow rate with decreased bile salt concentration, whereas low dose (20 – 200 mg/kg) increased the bile flow rate without altering biles salt concentration. Furthermore, the high dose of the extract increased biliary cholesterol output. Concurrent with increased biliary cholesterol secretion, *C. comosa* caused a decrease in plasma cholesterol level. These suggested that the increase in biliary cholesterol excretion was, at least, a factor which might have led to the lower level of plasma cholesterol (Pawinee Piyachaturawat et al., 1996). Subsequently, a phloracetophenone glucoside, [4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl) acetophenone] was isolated from the ethyl acetate and n-butanol extracts and has been identified as the choleretic principle of the plant (Apichart Suksamrarn et al., 1997). Study in rat model indicated that the aglycone part of compound, phloracetophenone (2,4,6-trihydroxyactophenone, THA), induced a dose-dependent increase in bile flow rate after a single intra-duodenal administration of THA at doses of 10 – 150 mg/kg. The increase in bile flow was associated with increased biliary secretion of bile acid, decreased secretion of cholesterol and phospholipid, and lowered bile lithogenic index. THA at a dose of 100 mg/kg induced a maximal increase of bile flow rate and bile acid output. A considerable decreased in plasma cholesterol was also observed which may attribute to the great choleretic activity with enhancement of biliary bile acid secretion. Among 14 acetophenone analogues, 2,4,6-trihydroxyacetophenone possessed the most potent choleretic effect, which induced both high blood flow rate and a high bile salt output then led to lower plasma cholesterol levels in rats (Pawinee Piyachaturawat et al., 2000). Although, Pawinee Piyachaturawat and colleagues did not find the significant changed total hepatic cholesterol content but observed the significantly increased secretion of both bile acids and cholesterol into intestinal lumen after THA treatment in hypercholesterolemic male hamsters (Pawinee Piyachaturawat et al., 2002a). Correlate with the increasing in bile acid excretion, THA caused a seven-fold increase in hepatic cholesterol 7α-hydroxylase activity (Pawinee Piyachaturawat et al., 2002a).

2. Hypolipidemic effect

Hypolipidemic effect of *C. comosa* had been found in many studies. From the choleretic effect of *C. comosa* rhizome extract and it remarkably stimulated bile

secretion and cholesterol which consequently led to a decrease in plasma cholesterol (Pawinee Piyachaturawat et al., 1996). *C. comosa* had hypolipidemic action by decreased plasma lipid levels of both TG and cholesterol in rats (Pawinee Piyachaturawat et al., 1998). Subsequent study by administration of *C. comosa* ethyl acetate extract at a dose of 0 – 500 mg/kg/day for 7 days in hypercholesterolaemic hamsters, shared a decrease of both plasma cholesterol and TG levels in a dose-dependent manner (Pawinee Piyachaturawat et al., 1999). Additionally, THA enhanced biliary excretion of bile acids and decreased plasma cholesterol in rats (Apichart Suksamrarn et al., 1997; Pawinee Piyachaturawat et al., 1998). Also, administration of THA (300 – 600 μmol/kg) twice a day for 7 days decreased both plasma cholesterol and TG level in hypercholesterolaemic hamster (Pawinee Piyachaturawat et al., 2002a)

3. Anti-oxidative effect

Anti-oxidative effect of crude ethanol extract of *C. comosa* had been revealed recently. The results clearly demonstrated that 4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl) acetophenone possessed high antioxidant effect (Somchit Niumsakul et al., 2007).

4. Anti-inflammatory effect

Hexane extract of *C. comosa* had been found to possess a strong anti-inflammatory activity in activated microglia (Nattinee Jantaratnotai et al., 2006). Recently, it had been found that *C. comosa* hexane extract, ethanol extract and two diarylheptanoids (5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(1*E*)-1-heptene and 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1*E*)-1-heptene) of *C. comosa* significantly decreased the release of pro-inflammatory cytokines, TNF-α, IL-1β, from phorbol-12-myristate-13-acetate (PMA)-stimulated PBMC and U937 cells (Amorntus Sodsai et al., 2007). In PMA-stimulated U937 cells, both diarylheptanoids reduced the expression of TNF-α, suppressed expression of IκB kinase and the activation of NF-κB (Amorntus Sodsai et al., 2007).

4. Anti-atherosclerotic effect

Treatment with hexane extract of *C. comosa* at the dosage of 100 mg/kg/day for 3 months in hypercholesterolemic rabbits restored endothelium-mediated vascular responses from high cholesterol diet mediated impairment of endothelium dependent relaxation. In addition, 100 mg/kg/day of the *C. comosa* extract decreased platelet aggregation as compared to high cholesterol diet control group (Piyanee Rattanachamnong, 2008). These effects of *C. comosa* to restore endothelium-mediated vascular responses and decreased platelet aggregation supposed to decrease endothelial dysfunction and platelet aggregation after plaque rupture, respectively. In addition, *C. comosa* directly decrease atherosclerotic plaque formation (Piyanee Rattanachamnong, 2008; Yupin Sanvarinda et al., 2007).

Toxicological effects

Acute toxicity of THA was dependent on species and route of administration. For subacute toxicity study, THA at high doses (150 and 300 mg/kg/day) significantly increased plasma concentration of alanine and aspartate aminotransferase, bilirubin, blood urea nitrogen (BUN), and hepatic TG content (Pawinee Piyachaturawat et al., 2002b). Songpol Chivapat and colleagues investigated a subchronic toxicity of ethanolic extract of *C. comosa* rhizome in Wistar rats by administration of *C. comosa* ethanolic extract at the doses of 100, 200, 400 and 800 mg/kg/day for 90 days. The result showed that at the dose of 800 mg/kg/day, *C. comosa* caused a significant decrease of hematocrit, red blood cells and hemoglobin in male rats while female rats receiving the extract at 200 mg/kg/day possessed a significantly decrease of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) but a significant increase of platelet number at 800 mg/kg/day. In addition, at the highest dose (800 mg/kg/day), a significant increase of these following blood chemistry parameters were found: ALP, ALT, total protein and albumin. Triglyceride level was significantly decreased in male rats treated with 400 mg/kg/day and female rats treated with 200 and 400 mg/kg/day. Moreover, *C. comosa* extract at 200 mg/kg/day increased stomach weight while other higher doses increased liver weight as well as hyperplasia and hyperkeratosis of gastric epithelium (Songpol Chivapat et al., 2003).