CHAPTER I

INTRODUCTION

Cardiovascular disease is one of the causes of morbidity and mortality in the United States and worldwide (American Heart Association, 2009). World Health Organization (WHO) estimates that 17.5 million people around the world died from cardiovascular disease in 2005. This is representing 30 percent of all global deaths. If appropriate action is not taken, by 2015, an estimated 20 million people will die from CVD every year (World Health Organization, 2009). However, if all forms of major cardiovascular disease were eliminated, life expectancy would rise by almost 7 years (American Heart Association, 2009). In Thailand, cardiovascular disease is the leading causes of death. Estimated mortality rate of cardiovascular disease was 164 per 100,000 populations in 2004 (World Health Organization, 2009). Atherosclerosis is a major cause of cardiovascular disease. Epidemiologic studies have identified various risk factors for atherosclerosis, these risk factors might be associated with lipid factors and non-lipid factors such as hypertension, diabetes, cigarette smoking, menopause and male (NCEP-ATPIII, 2001). For lipid factors, high levels of low density lipoprotein-cholesterol (LDL-C) have emerged as one of the strongest risk factor of atherosclerosis. The oxidation of LDL is the key factor of atherosclerosis. Oxidized LDL (oxLDL), interacts with the endothelial layer, attaches firmly to the endothelium, migrates into the subendothelial space, and then accumulates into the macrophage. After that, macrophage becomes foam cell which is an early state of atherosclerosis (Keaney, 2000; Stocker and Keaney, 2004). Recently, atherosclerosis is recognized as chronic inflammatory disease. Degraded products of oxLDL including malondialdehyde and 4-hydroxynonenal with anionic valence have strong immunogenic properties which activate immune mechanisms and inflammation after uptake of oxLDL by macrophage. The inflammation reactions are complex communication response between immune cells (Blasi, 2008). Therefore, cytokines, a group of protein associated with inflammation, are now believed to be related to atherosclerosis.

Cytokines are groups of protein cell regulators including lymphokines, monokines, interleukins (IL), interferon (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. IL-1β, TNF (tumor necrosis factor)-α, and monocyte chemotactic protein (MCP)-1 are prominent pro-inflammatory cytokines in atherosclerosis. In contrast, IL-10 and TGF (transforming growth factor)-β are recognized as anti-inflammatory cytokines in atherosclerosis. IL-1β mediates loss of endothelium-dependent relaxation (Chamberlain et al., 2009) and increases intercellular cell adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 gene expression in vascular smooth muscle cell which enhanced leukocyte adhesion to vascular smooth muscle cell (Wang et al., 1995). The second pro-inflammatory cytokine, MCP-1, triggers firm adhesion of monocytes to vascular endothelium (Luscinskas et al., 2000) and stimulates macrophage infiltration into the arterial wall (Namiki et al., 2002). Besides the chemoattracting properties, MCP-1 exhibits various deleterious effects 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 third pro-inflammatory cytokine, TNF-α, induces the expression of adhesion molecule which supports leukocyte attach to endothelial cells (Cavender, Saegusa, and Ziff, 1987) and plays a role in chemotactic activity (Ming, Bersani, and Mantovani, 1987). TNF-α is a potent stimulation of several matrix metalloproteinase (MMPs) (Rajavashisth et al., 1999) and plasminogen activator inhibitor (Galis et al., 1995). In the other hand, anti-inflammatory cytokine, IL-10, inhibits antigen-presentation capacity of macrophages and dendritic cells, blocks cytokines production including IL-1β, IL-6, IL-8, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor (G-CSF) in lipopolysaccharide (LPS)-induced monocytes (de Waal Malefyt et al., 1991). IL-10 shows prominent roles in inhibition of T helper cell (T_H) 1 immune responses which aggravate atherosclerotic lesion progression 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). In addition to inhibition of cytokine expression and releasing, IL-10 prevents atherosclerosis plaque degradation from

MMP-9 released by IL-18-stimulated or unstimulated peripheral blood mononuclear cell (PBMC) and enhances the secretion of tissue inhibitor of metalloproteinases-1 (TIMP-1) which counteracts MMPs effects (Nold et al., 2003). Furthermore, anti-inflammatory cytokine TGF-β has been 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). In macrophage, numerous gene expressions such as MMP-12, iNOS, pro-inflammatory cytokines including MCP-1 induced by LPS are suppressed by TGF-β (Singh et al., 2002). Moreover, TGF-β is able to reduce inflammatory responses by inhibition of nitric oxide and superoxide radicals which involve in inflammatory response but increase anti-inflammatory IL-10 expression (Maeda et al., 1995). In cholesterol uptake and efflux to macrophage, TGF-β attenuates the expression of scavenger receptor (SR), the cell-surface membrane proteins which internalize modified lipoprotein and then initiate transformation of macrophage to be foam cell. In the other hand, TGF-β increases the expression of ATP-binding cassette (ABC) transporter including ABCA1, ABCG1 that stimulate macrophage cholesterol efflux (Panousis, Evans, and Zuckerman, 2001). TGF-β stimulates the synthesis of proteoglycans and extracellular matrix proteins in vascular smooth muscle (Grainger et al., 1994). It also stimulates the expression of the interleukin-1 receptor antagonist (IL-1RA), an anti-inflammatory cytokine in IL-1 superfamily (Di Febbo et al., 1998).

Previous studies demonstrate that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins effectively inhibit cholesterol biosynthesis leading to decrease circulating LDL-C concentration. In addition, statins also increase HDL-C (high density lipoprotein-cholesterol) concentration and decrease triglyceride (TG) concentration (NCEP-ATP, 2001). In clinical studies, statins treatment not only decreases LDL-C but also reduces morbidity and mortality in hypercholesterolaemic patients with ischemic heart disease (Scandinavian Simvastatin Survival Study Group, 1994). These may be resulted from pleiotropic effect of statins, various effects which do not involve cholesterol lowering effect such as anti-oxidation and anti-inflammation. The effect of simvastatin to reduce inflammation in aorta can be visualized by using ^{18}fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) (Tahara et al., 2006). Simvastatin was found to reduce pro-inflammatory cytokines, IL-6, IL-8, and MCP-1, expression in hypercholesterolemic patients

(Rezaie-Majd et al., 2002; Montecucco et al., 2009) and to increase anti-inflammatory cytokine IL-10 in unstable angina patients (Li et al., 2006). In the other hand, it has been found that simvastatin may cause some adverse effects such as myalgias, myopathy and rhabdomyolysis (Bays, 2006). Adverse effects of simvastatin on the liver including cholelithiasis, cholecystitis, cholestatic jaundice, and liver failure are found in a small percentage of patients (Kiortsis et al., 2007). However, elevation of liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are commonly found (Cohen, Anania, and Chalasani, 2006). ALT is also known to be strongly associated with cardiovascular disease (Alkhouri et al., 2009). Although aminotransferases are extremely useful to detect liver function, elevations in aminotransferase levels from statins are not always indicative of liver damage or dysfunction (Cohen, Anania, and Chalasani, 2006). Besides serum liver enzyme used to investigate liver inflammation, cytokines are key mediators to control intracellular signaling pathways in hepatocytes, hepatocyte stellate cells, Kupffer cells leading to progression of inflammation in liver and induce acute and chronic liver injury (Tacke, Luedde, and Trautwein, 2009) which could be efficient indicators of liver injury and reversibility of liver damage (Lacour et al. 2005).

Curcuma comosa Roxb. is a plant in family Zingiberaceae. It is an indigenous plant of Thailand with a common name in Thai as Wann 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* include: (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) (Apichart Suksamrarn et al., 1994, 1997). Besides estrogenic effects, pharmacological effects of *C. comosa* which related to cardiovascular disease have been revealed. The choleretic effect of *C. comosa* rhizome extract had been recently investigated. It remarkably stimulated bile secretion and enhanced biliary excretion of bile salt and

cholesterol which consequently led to a decrease in plasma cholesterol (Pawinee Piyachaturawat, Gansar, and Apichart Suksamrarn, 1996). The hypolipidemic effect of *C. comosa* from ethyl acetate extract has been shown to effectively decrease LDL, TG and increased HDL (Pawinee Piyachaturawat et al., 1999). Also, several studies reported that *C. comosa* possessed strong anti-inflammatory activity (Nattinee Jantaratnotai et al., 2006; Amorntus Sodsai et al., 2007), anti-oxidative effect (Somchit Niumsakul et al., 2007; Cheerana Yomchot et al., 2008), and reduced atherosclerotic plaque formation and attenuated platelet aggregation (Piyanee Rattanachamnong, 2008).

C. comosa is an interesting plant with many pharmacological effects related to cardiovascular disease such as choleretic effect, hypolipidemic effect, anti-inflammatory effect, anti-oxidative effect, and anti-plaque formation. Thus, *C. comosa* is potential to be developed for medicinal purposes relating to cardiovascular disease. Effect of *C. comosa* on pro- and anti-inflammatory cytokines in aorta and liver of hypercholesterolemic animals would provide a molecular mechanism elaborating the protective effect of this plant against cardiovascular disease without adverse effect of liver toxicity found in simvastatin. Therefore, the aim of this study was to investigate effect of *C. comosa* rhizome on the expression of pro- and anti-inflammatory cytokines in rabbits fed with high cholesterol diet compared to those of the rabbits fed with high cholesterol diet alone and high cholesterol diet accompanying with a lipid lowering drug, simvastatin.

Hypothesis

C. comosa rhizome decreases pro-inflammatory cytokines and increases anti-inflammatory cytokines in abdominal aorta and liver of rabbits fed with high cholesterol diet.

Study design and process

1. Primer design
2. Tissue preparation
3. RNA extraction
4. Reverse transcription
5. Determination of cytokines expression
6. Data analysis

Anticipated benefits from the study

An information regarding the effects of *C. comosa* rhizome on cytokines expression in abdominal aorta and liver compared to simvastatin, the known medicine using in cardiovascular disease, will provide an information on the potential of this plant to be used in cardiovascular disease in the future.