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Effect of Andrographolide on Foam Cell Formation at the Initiation Stage of Atherosclerosis

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Abstract

Atherosclerosis is a chronic inflammatory disease that is caused by multiple processes. Inflammation is the main mechanism underlying the pathogenesis of atherosclerosis. The initiation stage of atherosclerosis is characterized by the formation of foam cells. Andrographolide is a compound that has an anti-inflammatory effect that is expected to be used as an anti-atherosclerosis. The purpose of this study was to evaluate andrographolide effect on foam cell formation at the initiation stage of atherosclerosis. The study was conducted on 27 rats divided into 3 groups (n=9). Group 1 was given a normal diet. Group 2 was given an atherogenic diet (vitamin D3 700,000 IU/kg on the first day and 2% cholesterol, 5% goat fat, 0.2% cholic acid and standard diet up to 100% for 2 days). Group 3 was given an atherogenic diet and andrographolide 40 mg/kg. The andrographolide effect on foam cell formation was assessed by histopathologic examination using hematoxylin eosin staining. The results showed that the number of foam cells was increased significantly in atherogenic diet-fed rats compared to normal diet-fed rats (82.33 + 13.10 vs. 5.33 + 1.73; P<0,05). Andrographolide reduced this number remarkably (82.33 + 13.10 vs. 7.44 + 1.62; P<0,05). In conclusion, andrographolide inhibits the formation of foam cells at the initiation stage of atherosclerosis. Thus andrographolide can be potentially developed as an anti-atherosclerosis.

Keywords: andrographolide, foam cell, atherosclerosis

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1. Introduction

Atherosclerosis is a chronic inflammatory disease that is caused by multiple processes. Inflammation is the main mechanism underlying the pathogenesis of atherosclerosis [1]. The early stage of atherosclerosis begins with the entry of low-density lipoprotein (LDL) into the intima of artery, especially in segments where hemodynamic disturbance occurs. The LDL will undergo oxidation to oxidized LDL (ox-LDL) which will activate

endothelial cells. Activated endothelial cells express leukocyte adhesion molecules such as Vascular Cell Adhesion Molecule 1 (VCAM-1) on the surface of the arteries. VCAM-1 then interacts with monocytes in the circulation [2].

Monocytes that have been attached to vascular endothelial cells then migrate into the arterial intima layer mediated by Monocyte Chemoattractant Protein-1 (MCP-1) [2]. Monocytes will then differentiate into macrophages in response to Macrophage-Colony Stimulating Factor (M-CSF) [3, 4]. These macrophages then devour excessive cholesterol via scavenger receptors which will lead to foam cell formation [4, 5]. Formation of foam cells that occurs in the initial stages of atherogenesis is a major hallmark of atherosclerotic disease [6].

Andrographolide is an active compound contained by Andrographis paniculata. Andrographis paniculata (Acanthaceae) is known as sambiloto (Indonesia), kalmegh (India) and chuanxinlian (China), which is used as an herbal medicine in Asia [7]. Based on previous studies andrographolide has been shown to have pharmacological effects as anti-inflammatory through its influence on cytokines that play a role in the inflammatory process [7-11]. With the discovery of anti-inflammatory role of andrographolide, its potential effect in inflammation has gradually attracted much attention, especially in atherosclerosis. The purpose of this study was to evaluate andrographolide effect on foam cell formation at the initiation stage of atherosclerosis.

2. Materials and Methods

2.1. Materials

Andrographolide (>98% purity) was purchased from Andalas Sitawa Fitolab (Padang, Indonesia). Vitamin D3 and cholic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Experimental animals

Twenty-seven healthy male Wistar rats (purchased from School of Pharmacy, Universitas Riau, Pekanbaru, Indonesia), 150-200 g body weight, 10 weeks of age were placed in cages individually in a room with proper ventilation, room temperature between 20–26 °C, and allowed for food and water ad libitum. Lighting of the room was regulated light and dark alternately for 12 hours. Animals underwent an acclimatization period at least 7 days before use in our study. During the acclimatization period the rats got a normal

diet. Rats were treated in accordance with the Helsinki convention. Ethical approval was obtained from the Ethical Review Board for Medicine & Health Research, Faculty of Medicine, Universitas Riau (No:457/UN.19.5.1.1.8/UEPKK/2017).

2.3. Experimental design

Rats were randomly divided into 3 groups (n=9), Group 1 was given a standard diet. Group 2 was given an atherogenic diet (vitamin D3 700.000 IU/kg on the first day followed by 2% cholesterol, 5% goat fat, 0.2% cholic acid and standard diet up to 100% for 2 days) to induce the initiation stage of atherosclerosis [12-14]. Group 3 was given an atherogenic diet and treated with andrographolide 40 mg/kg. Andrographolide was given orally once a day using a gastric tube.

2.4. Evaluation of foam cell formation

At the end of the experiment, the rats were sacrificed with ether anesthesia. Subsequently, abdominal aortic tissues were rapidly excised. We fixed the aortic sample in 10% neutralized formaldehyde in 0.1 M phosphate buffer and embedded in paraffin. A hematoxylin and eosin (H&E) staining was performed to count the number of foam cells in the abdominal aortic in rats. Foam cells were calculated at 9 fields of view with ×400 magnification. Images were obtained using a light microscope equipped with a digital camera (Leica, Wetzlar, Germany) connected to a PC monitor. Slides were examined by two independent pathologists from Department of Pathology, Faculty of Medicine, Universitas Riau.

2.5. Statistical analysis

Data are presented as mean ± SEM. All results were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. $P<0.05$ was considered statistically significant.

3. Results

The H&E staining showed that in Group 1 receiving normal diet and Group 3 receiving an atherogenic diet and treated with andrographolide demonstrated few foam cells (Figs. 1A and 1C), while in Group 2 receiving an atherogenic diet showed abundance of foam

cells in the aortic lesion (Fig. 1B). These results demonstrated that the administration of an atherogenic diet induced foam cell formation and andrographolide treatment inhibited the accumulation of these cells in aortic lesion.

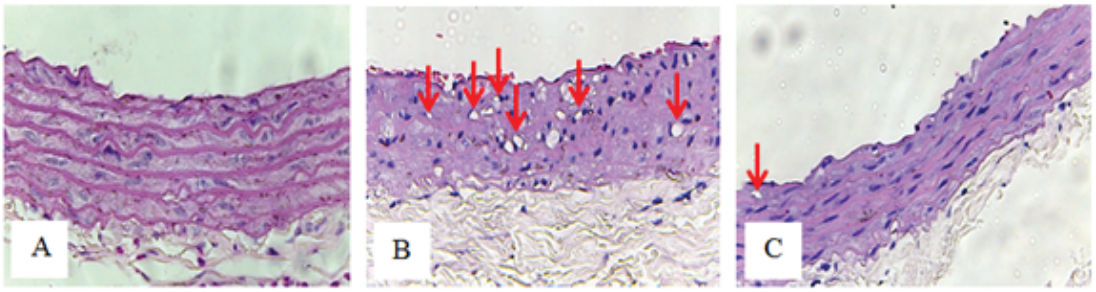

Figure 1: Photomicrographs showing histological sections of aorta abdominal of rats. A) Group 1 was given a standard diet, B) Group 2 was given an atherogenic diet, C) Group 3 was given an atherogenic diet and treated with andrographolide 40 mg/kg, at magnification of ×400 stained with H&E. The red arrow indicated foam cell.

Fig. 2 shows the number of foam cells among different experimental groups. Atherogenic diet-fed animals exhibit increased number of foam cells in the aortic lesions compared to control rats ($P<0.05$). Andrographolide treatment significantly reduced the accumulation of foam cells in the aortic lesions ($P<0.05$).

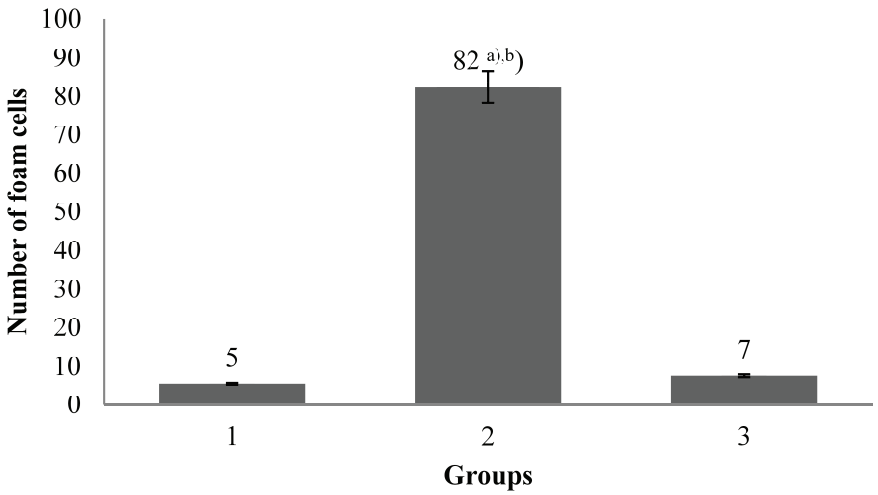

Figure 2: Number of foam cells among different experimental groups.

Group 1 receiving normal diet; Group 2 receiving an atherogenic diet; Group 3: receiving an atherogenic diet and treated with andrographolide 40 mg/kg. Data are presented as mean + SEM (N=9). Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) and Bonferroni's post hoc test for average comparison on SPSS 17.0. a)$P<0.05$ vs. Group 1. b)$P<0.05$ vs. Group 3.

4. Discussion

During the recent years, many researchers have paid attention to phytochemicals, which are regarded as important sources for drug development [15]. Andrographolide, a diterpenoid lactone, is the main ingredient of Andrographis paniculata [16]. Andrographolide is well absorbed and non-toxic even at very high doses in animals and is well tolerated by humans with no serious side effects at doses 1 to 2 mg/kg/day. Andrographolide shown gastro-protective and ulcer preventive effects, which combined with its anti-inflammatory effects could make it a safe alternative to traditional NSAIDs [7]. In this study, we found that andrographolide may reduce foam cell formation in the early stages of atherosclerosis.

The formation of foam cells involves various processes such as taking ox-LDL, cholesterol esterification and cholesterol efflux. Ox-LDL uptake by macrophages is carried out through phagocytosis and pinocytosis processes mediated by CD36 and SR-A. About 75-90% of ox-LDL uptake by macrophages was carried out through both receptors [5, 17]. Manning-Tobin et al. reported the results of his study which showed that SR-A suppression can prevent the formation of atherosclerotic lesions [18], while research conducted by Xie et al. showed that down-regulation of CD36 can inhibit foam cell formation [19].

The process of cholesterol esterification involves Acyl coenzyme A: cholesterol acyltransferase-1 (ACAT1) and neutral cholesteryl ester hydrolase (nCEH) [20]. The level of cholesterol esterification determines the number of macrophages that will turn into foam cells [21]. Xu et al. showed ACAT1 inhibition can suppress foam cell formation and inhibit the development of atherosclerosis [22].

The process of cholesterol efflux involves the ATP-binding cassette (ABC) transporter A1 (ABCA1), ABCG1 and BI-receptor scavenger (SR-BI) [23, 24]. Disruption in the process of cholesterol efflux results in cholesterol accumulation in macrophages [25], whereas ABCA1 stimulation can inhibit foam cell formation [26].

The balance between cholesterol uptake, cholesterol esterification and cholesterol efflux plays a role in preventing lipid accumulation in macrophages [27]. At the stage of atherosclerosis initiation there is an increase in CD36 and SR-A expression, an increase in cholesterol esterification due to an increase in ACAT1 levels or a decrease in nCEH levels and a decrease in cholesterol efflux due to a decrease in the expression of ABCA1, ABCG1 and SR-BI. This condition causes the accumulation of cholesterol esters in the macrophages which will result in the formation of foam cells [28]. The progression of atherosclerosis depends on the accumulation of monocytes on the walls of the arteries,

the more monocytes/macrophages accumulate, the more foam cells are formed so that the development of atherosclerotic lesions will also accelerate [29, 30].

The initiation stage of atherosclerosis is characterized by the formation of foam cells [28]. Foam cells found at the initiation stage of atherosclerosis are derived from monocytes found in the circulation [31]. Monocytes that have been attached to vascular endothelial cells then migrate into the intima layer of arteries [2]. The mechanism of andrographolide in inhibiting foam cell formation is thought to be through the inhibition of monocyte migration into the arterial wall.

5. Conclusion

In conclusion, andrographolide could inhibit the formation of foam cells at the initiation stage of atherosclerosis. Thus andrographolide is very potential to be developed as anti-atherosclerosis.

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Disclosure

The authors declare no conflict of interest.

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