BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 19(4), 472-476 (2011)

Effect of Yacon on Platelet Function in Hypercholesterolemic Rabbits

Yong Lim[1,a], Dong-Ju Son[2,a], Yun-Bae Kim[3], Bang-Yeon Hwang[2], Yeo-Pyo Yun[2,*] and Seock-Yeon Hwang[4,*]

[1]Department of Clinical Laboratory Science, Dong-eui Univerisity, Busan 614-714,
[2]College of Pharmacy, [3]Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju 361-763,
[4]Department of Biomedical Laboratory Science, Daejeon University, Daejeon 300-716, Republic of Korea

Abstract

Hypercholesterolemia indirectly increases the risk of arterial and venous thrombosis by enhancing the ability of platelets to aggregate. Yacon (*Smallanthus sonchifolius*) is composed of fructooligosaccharides, proteins, minerals and phenolic compounds, and has potential benefits for the management of diabetes. This study investigated whether the consumption of yacon in the diet inhibits platelet aggregation under hypercholesterolemic conditions. Male New Zealand white rabbits were fed one of five dietary interventions: a normal control diet, 0.5% cholesterol diet, 0.5% cholesterol diet+a low dose of yacon (0.5 g/kg body weight given orally each day), 0.5% cholesterol diet+a high dose of yacon (2.5 g/kg body weight given orally each day), or a 0.5% cholesterol diet+lovastatin (2 mg/kg body weight given orally each day). After 8 weeks, blood was collected to measure the amount of collagen- and thrombin-induced platelets present. Yacon inhibited the platelet aggregation induced by low doses of agonists (0.5 μg/ml collagen and 0.02 units/ml thrombin) in a concentration-dependent manner. In addition, yacon concentration-dependently inhibited collagen-induced arachidonic acid liberation. Moreover, *n*-hexane, chloroform and ethyl acetate fractions showed a marked and selective inhibition of the platelet aggregation induced by collagen, again in a dose-dependent manner. These fractions, especially that of chloroform, significantly suppressed platelet aggregation. The results of this study demonstrate that when yacon is added to a cholesterol-enriched diet, cholesterol-induced platelet aggregation returns to control levels. This may also be beneficial in preventing atherosclerosis and reducing risk factors for coronary artery disease and stroke.

Key Words: Antiplatelet, Yacon, Hypercholesterolemia, Thrombosis

INTRODUCTION

Hypercholesterolaemia and platelet hyper-reactivity are associated conditions and closely participate in the development of atherosclerosis-related thrombotic events (Fuster *et al.*, 1992; Notarbartolo *et al.*, 1995). Platelets are surrounded by low-density lipoproteins (LDL) and high-density lipoproteins (HDL) in circulating blood and they may also interact with circulating oxidatively-modified LDL (Sevanian *et al.*, 1996; Sevanian *et al.*, 1997; Holvoet *et al.*, 1998; Tanaga *et al.*, 2002; Kovanen and Pentikainen, 2003; Sanchez-Quesada *et al.*, 2004). The interaction of platelets with LDL and oxidized LDL may explain the higher platelet aggregability associated with hypercholesteremia, diabetes and cigarette smoking (Willoughby *et al.*, 2002; Patrono *et al.*, 2005; Watala, 2005). Moreover, upon rupture of vulnerable lipid-rich plaques, platelets become exposed to oxidatively- and enzymatically-modified LDL and their degradation products, which accumulate in the lipid-rich plaque core (Bocan *et al.*, 1986), the most thrombogenic part of atherosclerotic plaques (Fernandez-Ortiz *et al.*, 1994). Platelet activation by this plaque material could be important in arterial thrombus formation, leading to acute coronary syndromes (angina pectoris, myocardial infarction) or cerebral ischemia (TIA, stroke).

Yacon (*Smallanthus sonchifolius*) is a plant originally cultivated in South America, and the fresh root is eaten like a fruit in this area. Yacon roots are sometimes used for home cooking, but the plant has not been commonly adopted as a foodstuff because of its tendency to decay quickly and to show rapid browning of the juice or injured tissues. The browning may be caused by a condensation reaction of polyphenols with amino compounds (Yabuta *et al.*, 2001) and the enzy-

www.biomolther.org

Open Access **http://dx.doi.org/10.4062/biomolther.2011.19.4.472**

pISSN: 1976-9148 eISSN: 2005-4483

Received Apr 29, 2011 **Revised** Sep 6, 2011 **Accepted** Sep 8, 2011

***Corresponding Author**
E-mail: syhwang@dju.kr (Hwang SY), ypyun@chungbuk.ac.kr (Yun YP)
Tel: +82-42-280-2902 (Hwang SY), +82-43-261-2821 (Yun YP)
Fax: +82-42-280-2904 (Hwang SY), +82-43-268-2732 (Yun YP)
[a]These authors contributed equally to this work.

matic polymerization of polyphenols. Yacon juice contains 850 ppm of polyphenolic compounds, which generally have antioxidant activity, and, in addition to these, chlorogenic acid and tryptophan have been reported to be major antioxidants in yacon (Yan *et al.*, 1999). Previously, we found that yacon extract showed a useful for management of hyperglycemia and diabetic nephropathy (Park *et al.*, 2009).

In this study, we evaluated the inhibitory properties of yacon extract on the increased platelet aggregation observed under hypercholesterolemic conditions. Furthermore, washed rabbit platelets were preincubated with various concentrations of each of the fractions derived from yacon methanolic extract (50-200 μg/ml) to examine the extent of the inhibitory effect of an agonist (collagen) on platelet aggregation.

MATERIALS AND METHODS

Reagents and chemicals

Cholesterol, lovastatin and 1,2-diacylglycerol (1,2-DAG) were purchased from Sigma Chemical Co. (St Louis, MO, USA), whilst collagen, thrombin and arachidonic acid (AA) were purchased from Chrono-Log Co. (Havertown, PA, USA). [^{3}H]-AA (100 μCi/mmol) was procured from PerkinElmer Life Sciences Inc. (Boston, MA, USA), the anaesthetic Zoletil was from Virbac Laboratories Ltd. (Carros Cedex, France), and the other chemical reagents were from the Sigma Chemical Co.

Preparation of yacon tuber extract

Plant materials were purchased from Bonghwa (Gyeongbuk, Korea) and authenticated by Dr. Hwang, a botany professor at the College of Pharmacy, Chungbuk National University (Cheongju, Korea) (Park *et al.*, 2009). Yacon tubers were washed with water and dried under sunlight. For extraction, the dried tubers were soaked overnight in 50% ethanol at room temperature and the extract was sonicated three times for 5 hours at 60°C and filtered through filter paper (Whatman No.1). The extract was then concentrated in a rotary evaporator under reduced pressure and freeze-dried to a powder that was later dissolved in water at a concentration of 100 mg/ml for further use.

Experimental design

A total of 30 rabbits (New Zealand white, male, 2.0 ± 0.2 kg) were purchased from Samtako Experimental Animal Breeding Co., Ltd. (Osan, Kyeonggi, ROK). They were provided with solid rabbit food and sterilized, distilled water *ad libitum*, and kept at a constant temperature (23 ± 2°C) and relative humidity (55 ± 10%). Ventilation operated 12 times per hour and the animals were maintained on a 12-hour light (150-300 lux)/12-hour dark cycle. They were housed under these conditions for at least two weeks after delivery from the breeding company before the experiment commenced. For the experimental phase, a single rabbit was placed in a cage measuring 380 *W* ×490 *L*×350 *H* mm. Animals were randomly allocated to one of five treatment groups with the same sample size in each. Groups were fed either a normal chow diet (NCD), a high-cholesterol diet (HCD), a low dose of yacon (0.5 g/kg body weight), a high dose of yacon (2.5 g/kg body weight), or lovastatin (2 mg/kg body weight). Yacon and lovastatin were given orally each day. All animals except those in the NCD group were fed a HCD containing 2% corn oil and 0.5% cholesterol for 4 weeks to induce a stable state of hypercholesterolemia. Yacon and lovastatin were fed for 8 weeks after the total blood cholesterol level reached 910-940 mg/dl. Animal studies were approved by the Chungbuk National University Animal Care and Use Committee and experiments were performed under its control and in accordance with the Institutional Guidelines of Chungbuk National University, Korea, and the requirements for the maintenance of SPF status.

Platelet aggregation analysis

Platelet aggregation analysis was performed using washed rabbit platelets isolated from each experimental group of animals at the end of the experimental period. Preparation of the washed platelets and the platelet aggregation analysis were performed using previously described approaches (Hwang *et al.*, 2008). Platelet aggregation studies were performed by a standard turbidometric method using an aggregometer (Chrono-Log Co., Havertown, PA, USA). Aggregation, which was induced by a low dose of collagen (0.5 μg/ml) and thrombin (0.02 unit/ml), was expressed as an increase in light transmission. The rate of aggregation was obtained from the maximum aggregation induced by the respective agonist at the specified concentration using the equation: inhibition rate = maximum aggregation rate (MAR) of vehicle-treated platelet-rich plasma (PRP) - MAR of sample-treated PRP/MAR of vehicle-treated PRP×100.

Arachidonic acid liberation measurement

The effect of yacon on arachidonic acid (AA) liberation stimulated by collagen in [^{3}H]-AA-labeled hypercholesterolemic rabbit platelets was assayed using a previously-published method (Hwang *et al.*, 2008). In brief, PRP prepared from each experimental group of rabbits was preincubated with [^{3}H]-AA (1 μCi/ml) at 37°C for 1.5 hours, and then washed with HEPES solution. The [^{3}H]-AA-labeled platelets (4×10^8 cells/ml) were pretreated with 100 μM BW755C, a cyclooxygenase (COX) and lipoxygenase (LOX) inhibitor, and then aggregation was stimulated by collagen (0.5 μg/ml) for 3 minutes in the presence of 1 mM $CaCl_2$. The reaction was terminated by the addition of chloroform/methanol/HCl (200/200/1, v/v/v). Lipids were extracted and separated by TLC on silica gel G plates using a petroleum ether/diethyl ether/acetic acid (40/40/1, v/v/v) development system. The area corresponding to each lipid was scraped off and the radioactivity was determined by liquid scintillation counting.

Statistical analysis

The experimental results were expressed as mean ± SEM A one-way analysis of variance (ANOVA) was used for multiple comparisons followed by the Dunnett test. A probability value of $p<.05$ was considered to be statistically significant.

RESULTS

Effect of yacon on platelet hyper-activation under hypercholesterolemia

Platelet aggregation *ex vivo* was evaluated in response to weak stimulation using washed PRP from each group. Platelets from rabbits of the normal chow diet (NCD) were similar in shape and aggregation to those of animals fed diets with a low dose of collagen (0.5 μg/ml) (Fig. 1A first column) or

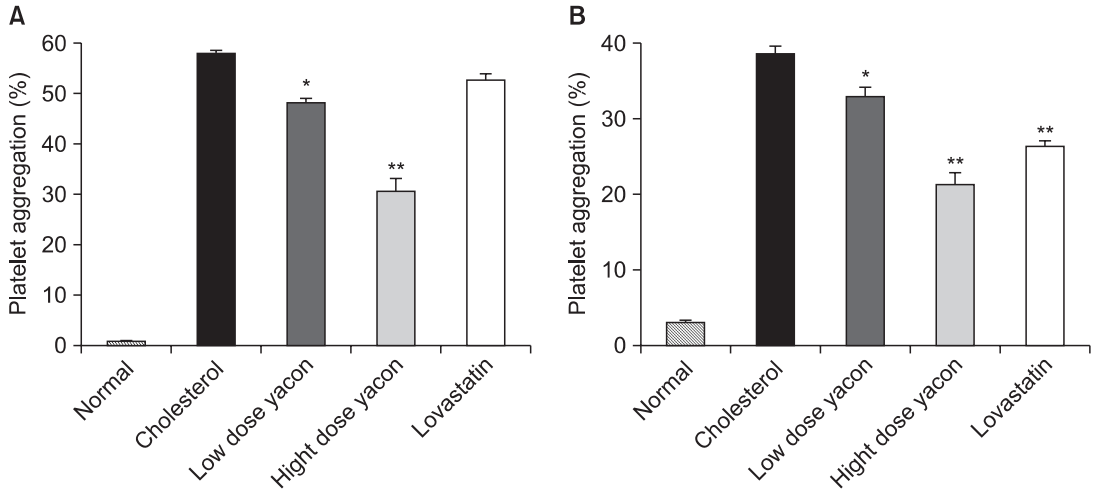

Fig. 1. The inhibitory effect of yacon administration on hypercholesterolemic platelet aggregation. Platelet rich plasma from each experimental group was preincubated with 1 mM $CaCl_2$ for 3 min, and stimulated in an aggregometer with 0.5 μg/ml collagen (A) or 0.02 units/ml thrombin (B). The concentrations of collagen and thrombin provided low stimulatory doses encouraging platelet aggregation. The aggregation percentages were expressed as the percentage of maximum aggregation induced by the respective agonists. Values are expressed as mean ± SEM (n=6). *p<0.05 and **p<0.01 indicate statistically significantly differences with respect to rabbits fed a hypercholesterolemic diet.

thrombin (0.02 units/ml) (Fig. 1B first column). Aggregation was potently enhanced by the hypercholesterolemic diet, with collagen- and thrombin-induced platelet aggregation percentages of 58.00 ± 2.16% (Fig. 1A, second column) and 39.86 ± 4.81% (Fig. 1B, second column), respectively. The yacon-treated group showed a significant reduction in aggregation under these conditions (Fig. 1). Lovastatin inhibited platelet aggregation by approximately 26.44 ± 2.15% (Fig. 1B fifth column) when thrombin was used as an agonist, whereas it did not show a significant inhibitory action in the presence of collagen (Fig. 1A, fifth column). These results demonstrate that yacon was more effective than lovastatin when used as an inhibitor of platelet hyper-activation enhanced by hypercholesterolemia.

Effect of yacon on collagen-induced AA formation

The antiplatelet effect of yacon on platelet membrane phospholipid metabolites and the liberation of AA when stimulated by collagen were investigated. This was achieved using 0.5 μg/ml collagen and [^{3}H]-AA-labeled rabbit platelets from each experimental group. The liberation of [^{3}H]-AA from activated platelets was significantly increased in the 0.5% cholesterol diet group compared to that from platelets of the group fed the control diet. Yacon concentration-dependently inhibited collagen-induced AA liberation from [^{3}H]-AA prelabeled rabbit platelets by 19.9% and 55.2% at the concentrations of 0.5 and 2.5 g/kg, respectively. Lovastatin also significantly suppressed AA liberation (Fig. 2).

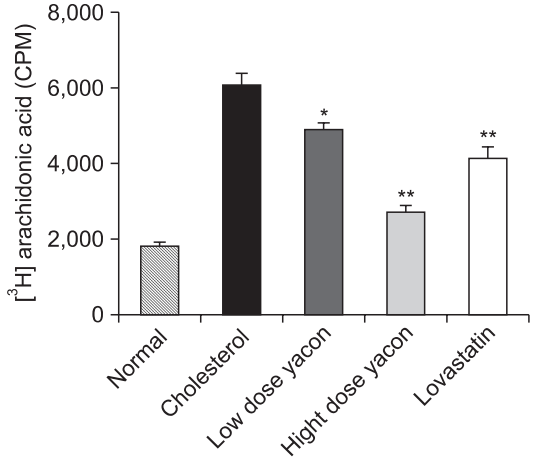

Fig. 2. Effect of yacon on collagen-induced AA formation in hypercholesterolemic platelets. [^{3}H]-AA-labeled platelets from each experimental group were incubated with 50 μM BW755C and 1 mM $CaCl_2$, and then stimulated with 0.5 μg/ml collagen for 3 min. The amount of [^{3}H]-AA liberated from activated platelets was determined as described in the Materials and Methods. Values are expressed as mean ± SEM (n=6). *p<0.05 and **p<0.01 indicate statistically significant difference with respect to rabbits fed a hypercholesterolemic diet.

Effect of fractions obtained from the yacon methanolic extract on platelet aggregation

Washed rabbit platelets were preincubated with various concentrations of each fraction derived from the yacon methanolic extract (50-200 μg/ml) and then exposed to collagen to elucidate the inhibitory effect of each fraction on platelet aggregation. As shown in Fig. 3, the chloroform fraction displayed the highest activity with a percentage inhibition at 50, 100 and 200 μg/ml of 28.0 ± 2.4, 56.9 ± 2.3 and 84.8 ± 1.8%, respectively (Fig. 3). In addition, the chloroform fraction did not show any cytotoxic effect on cell viability as assessed by WST-1 at the highest concentration used in this experiment (200 μg/ml) (data not shown). This suggests that the inhibitory effect of this fraction on platelet aggregation was not achieved through cytotoxicity.

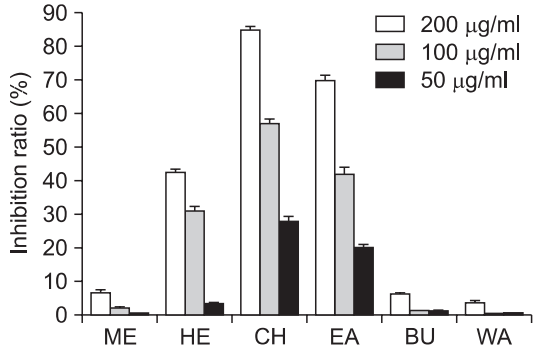

Fig. 3. Effect of fractions obtained from yacon methanolic extract on platelet aggregation. Washed rabbit platelets were incubated with various concentrations of fractions [methanol (ME), n-hexane (HE), chloroform (CH), ethyl acetate (EA), butanol (BU) and water (WA) (each 200 μg/ml)] from yacon methanolic extract. Platelet aggregation was induced by the addition of collagen (2 μg/ml). Each value represents the mean ± SEM (n=3).

DISCUSSION

Our data show that the administration of yacon prevents the excessive platelet aggregation associated with hypercholesterolemia and that this operates through the suppression of arachidonic acid (AA) liberation.

Platelets play a fundamental, life-saving role in haemostasis and blood clotting at sites of vascular injury. Under conditions of normal blood circulation, platelets cannot aggregate by themselves, but when blood vessels are damaged, they adhere to the disrupted surface and aggregate. Platelet hyperaggregability is associated with risk factors for atherothrombotic events and diseases such as hypercholesterolemia and hypertension. Hypercholesterolemia has been established as an independent risk factor in its own right for the development of atherosclerotic cardiovascular diseases. Changes in plasma lipoproteins affecting platelet function have been found in those experiencing hypercholesterolemia (Willoughby *et al.*, 2002). *In vitro* cholesterol depletion from the human platelet membrane decreases platelet sensitivity to agonists such as ADP, collagen, and thrombin, and reduces the release of thromboxane A_2 (TXA_2) and ADP (Bodin *et al.*, 2001; Grgurevich *et al.*, 2003; Quinton *et al.*, 2005; van Lier *et al.*, 2008). In contrast, *in vitro* cholesterol enrichment of human platelets results in hypersensitivity to agonists (Shattil *et al.*, 1975; Kramer *et al.*, 1982). These previous studies indicate that the cholesterol content of human platelets directly influences their activation, and suggest an important role for hypersensitive platelets in the development of thrombosis and atherosclerosis.

The present study examined firstly whether the administration of yacon reduces platelet aggregation in hypercholesterolemic rabbits fed a 0.5% cholesterol diet. Yacon administration potently inhibited platelet hyperaggregability induced by low doses of agonists (0.5 μg/ml collagen and 0.02 units/ml thrombin) with inhibition percentages of 45.5% (Fig. 1A) and 44.8% (Fig. 1B), respectively. Under hypercholesterolemic conditions, this inhibitory property of yacon was more effective than that of lovastatin (Fig. 1A and B). It was therefore hypothesized that the inhibitory property of yacon operates through the regulation of platelet function.

Since AA is a precursor of TXA_2, a potent inducer of platelet aggregation, prostaglandin endoperoxides and other eicosanoids, AA liberation is an important regulatory factor in platelet adhesion and aggregation. Pharmacological intervention in the arachidonate cascade is widely used as a therapy for hyperactive platelets and in the prevention of thromboembolic complications (Hashizume *et al.*, 1997; Nosal and Jancinova, 2001).

To identify the inhibitory mechanism of yacon on hypercholesterolemic platelet hyperaggregation, the study investigated the collagen-induced generation of AA. In the hypercholesterolemic animal model system used, yacon significantly suppressed AA liberation (Fig. 2A). Furthermore, washed rabbit platelets were preincubated with various concentrations (50-200 μg/ml) of each of the fractions derived from yacon methanolic extract. These were then exposed to collagen (0.5 μg/ml) to identify which fractions were responsible for the inhibitory effect of yacon on platelet aggregation. The chloroform fraction displayed the highest activity (Fig. 3) and this fraction may prove useful in the development of antithrombotic agents targeting the inhibition of the AA cascade. In conclusion, the study provides new evidence that fractions from yacon methanolic extract, especially the chloroform fraction, are capable of attenuating platelet aggregation. Identification of the bioactive constituents responsible for this antiplatelet effect is under investigation, together with a detailed examination of further inhibitory mechanisms of yacon.

Taken together, the present study suggests that the antiplatelet effect of yacon may, at least partly, be due to the inhibition of AA generation in hypercholesterolemic rabbit platelets. Therefore, the results suggest that the oral administration of yacon may be useful in the prevention of thrombi and atheroma formation in hypercholesterolemia.

ACKNOWLEDGMENTS

This work was supported by research funds of Daejeon University in 2009.

REFERENCES

Bocan, T. M., Schifani, T. A. and Guyton, J. R. (1986) Ultrastructure of the human aortic fibrolipid lesion. Formation of the atherosclerotic lipid-rich core. *Am. J. Pathol.* **123**, 413-424.

Bodin, S., Giuriato, S., Ragab, J., Humbel, B. M., Viala, C., Vieu, C., Chap, H. and Payrastre, B. (2001) Production of phosphatidylinositol 3,4,5-trisphosphate and phosphatidic acid in platelet rafts: evidence for a critical role of cholesterol-enriched domains in human platelet activation. *Biochemistry* **40**, 15290-15299.

Fernandez-Ortiz, A., Badimon, J. J., Falk, E., Fuster, V., Meyer, B., Mailhac, A., Weng, D., Shah, P. K. and Badimon, L. (1994) Characterization of the relative thrombogenicity of atherosclerotic plaque components: implications for consequences of plaque rupture. *J. Am. Coll. Cardiol.* **23**, 1562-1569.

Fuster, V., Badimon, L., Badimon, J. J. and Chesebro, J. H. (1992) The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N. Engl. J. Med.* **326**, 242-250.

Grgurevich, S., Krishnan, R., White, M. M. and Jennings, L. K. (2003) Role of in vitro cholesterol depletion in mediating human platelet aggregation. *J. Thromb. Haemost.* **1**, 576-586.

Hashizume, T., Nakao, M., Kageura, T. and Sato, T. (1997) Sphingosine enhances arachidonic acid liberation in response to U46619 through an increase in phospholipase A2 activity in rabbit platelets.

J. Biochem. **122**, 1034-1039.
Holvoet, P., Vanhaecke, J., Janssens, S., Van de Werf, F. and Collen, D. (1998) Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* **98**, 1487-1494.
Hwang, S. Y., Son, D. J., Kim, I. W., Kim, D. M., Sohn, S. H., Lee, J. J. and Kim, S. K. (2008) Korean red ginseng attenuates hypercholesterolemia-enhanced platelet aggregation through suppression of diacylglycerol liberation in high-cholesterol-diet-fed rabbits. *Phytother. Res.* **22**, 778-783.
Kovanen, P. T. and Pentikainen, M. O. (2003) Circulating lipoproteins as proinflammatory and anti-inflammatory particles in atherogenesis. *Curr. Opin. Lipidol.* **14**, 411-419.
Kramer, R. M., Jakubowski, J. A., Vaillancourt, R. and Deykin, D. (1982) Effect of membrane cholesterol on phospholipid metabolism in thrombin-stimulated platelets. Enhanced activation of platelet phospholipase(s) for liberation of arachidonic acid. *J. Biol. Chem.* **257**, 6844-6849.
Nosal, R. and Jancinova, V. (2001) Pharmacological intervention with platelet phospholipase A2. *Bratisl. Lek. Listy* **102**, 447-453.
Notarbartolo, A., Davi, G., Averna, M., Barbagallo, C. M., Ganci, A., Giammarresi, C., La Placa, F. P. and Patrono, C. (1995) Inhibition of thromboxane biosynthesis and platelet function by simvastatin in type IIa hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* **15**, 247-251.
Park, J. S., Yang, J. S., Hwang, B. Y., Yoo, B. K. and Han, K. (2009) Hypoglycemic effect of yacon tuber extract and its constituent, chlorogenic acid, in streptozotocin-induced diabetic rats. *Biomol. Ther.* **17**, 256-262.
Patrono, C., Falco, A. and Davi, G. (2005) Isoprostane formation and inhibition in atherothrombosis. *Curr. Opin. Pharmacol.* **5**, 198-203.
Quinton, T. M., Kim, S., Jin, J. and Kunapuli, S. P. (2005) Lipid rafts are required in Galpha(i) signaling downstream of the P2Y12 receptor during ADP-mediated platelet activation. *J. Thromb. Haemost.* **3**, 1036-1041.
Sanchez-Quesada, J. L., Benitez, S. and Ordonez-Llanos, J. (2004) Electronegative low-density lipoprotein. *Curr. Opin. Lipidol.* **15**, 329-335.
Sevanian, A., Bittolo-Bon, G., Cazzolato, G., Hodis, H., Hwang, J., Zamburlini, A., Maiorino, M. and Ursini, F. (1997) LDL- is a lipid hydroperoxide-enriched circulating lipoprotein. *J. Lipid. Res.* **38**, 419-428.
Sevanian, A., Hwang, J., Hodis, H., Cazzolato, G., Avogaro, P. and Bittolo-Bon, G. (1996) Contribution of an in vivo oxidized LDL to LDL oxidation and its association with dense LDL subpopulations. *Arterioscler. Thromb. Vasc. Biol.* **16**, 784-793.
Shattil, S. J., Anaya-Galindo, R., Bennett, J., Colman, R. W. and Cooper, R. A. (1975) Platelet hypersensitivity induced by cholesterol incorporation. *J. Clin. Invest.* **55**, 636-643.
Tanaga, K., Bujo, H., Inoue, M., Mikami, K., Kotani, K., Takahashi, K., Kanno, T. and Saito, Y. (2002) Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. *Arterioscler. Thromb. Vasc. Biol.* **22**, 662-666.
van Lier, M., Verhoef, S., Cauwenberghs, S., Heemskerk, J. W., Akkerman, J. W. and Heijnen, H. F. (2008) Role of membrane cholesterol in platelet calcium signalling in response to VWF and collagen under stasis and flow. *Thromb. Haemost.* **99**, 1068-1078.
Watala, C. (2005) Blood platelet reactivity and its pharmacological modulation in (people with) diabetes mellitus. *Curr. Pharm. Des.* **11**, 2331-2365.
Willoughby, S., Holmes, A. and Loscalzo, J. (2002) Platelets and cardiovascular disease. *Eur. J. Cardiovasc. Nurs.* **1**, 273-288.
Yabuta, G., Koizumi, Y., Namiki, K., Hida, M. and Namiki, M. (2001) Structure of green pigment formed by the reaction of caffeic acid esters (or chlorogenic acid) with a primary amino compound. *Biosci. Biotechnol. Biochem.* **65**, 2121-2130.
Yan, X., Suzuki, M., Ohnishi-Kameyama, M., Sada, Y., Nakanishi, T. and Nagata, T. (1999) Extraction and identification of antioxidants in the roots of yacon (Smallanthus sonchifolius). *J. Agric. Food. Chem.* **47**, 4711-4713.