Effects of Geiji-Bokryung-Hwan on eNOS, nNOS, Caveolin-1 and bFGF Protein Expressions and the Endothelial Cells of the Corpus Cavernosum in Hypercholesterolemic Rat

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We examine the effect of Geiji-Bokryung-Hwan(GBH) on erectile function in a rat model of hypercholesterolemic erectile dysfunction. GBH, a drug preparation consisting of five herbs of *Cinnamomi Ramulus* (Geiji), *Poria Cocos* (Bokryun), *Mountan Cortex Radicis* (Mokdanpi), *Paeoniae Radix* (Jakyak), and *Persicae Semen* (Doin) is a traditional Korean herbal medicine that is widely used in the treatment of atherosclerosis-related disorders. In this study, 3-month-old Sprague-Dawley rats were used. The 6 rats control animals were fed a normal diet and the other 18 rats were fed 1% cholesterol diet for 3 months. After 1 months, GBH was added to the drinking water of the treatment group of 12 rats but not the cholesterol only group of 6 rats. Of the 12 rats 6 received 30 mg/kg per day (group 1) and 6 received 60 mg/kg per day (group 2) of GBH. At 3 months erectile function was evaluated with cavernous nerve electrostimulation in all animals. Penile tissues were collected for electron microscopy, and to perform Western blot for endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), basic fibroblast growth factor (bFGF) and caveolin-1. Systemic arterial pressure was not significantly different between the animals that were fed the 1% cholesterol diet and the controls. Conversely erectile function was not impaired in the herbal medicine treated rats. Electron microscopy showed many caveolae with fingerlike processes in the cavernous smooth muscle and endothelial cell membranes in control and treated rats but not in the cholesterol only group of rats. Western blot showed differences among groups in protein expression for eNOS, nNOS, caveolin-1 and bFGF protein expression in penile tissue. Increased eNOS and nNOS protein expressions by high cholesterol diet were significantly decreased in group 1 and group 2. Interestingly, caveolin-1 and bFGF protein expression was significantly higher in groups 1 and 2 than in the cholesterol only and control groups.

Key words : Hypercholesterolemia, erectile dysfunction, endothelium cell, Geiji-Bokryung-Hwan (GBH), bFGF, eNOS, nNOS, bFGF, caveolin

Introduction

The pathogenesis of atherosclerosis involves endothelium dysfunction, infiltration of monocytes, activation of monocytes into macrophages, and smooth muscle cell proliferation.

Hypercholesterolemia is a contributing factor to erectile dysfunction. Every increase in total cholesterol level is associated with a 1.32 times higher risk of erectile dysfunction in men[2]. In studies of hypercholesterolemic rabbits impaired relaxation of cavernous smooth muscle in response to endothelium mediated and endothelium independent stimuli has been reported[3,4]. Electron microscopy of erectile tissue in hypercholesterolemic rabbits has demonstrated evidence of a focal atherosclerosis-like process. Reducing serum cholesterol level has been shown to restore the morphology of smooth muscle cells and erectile function[3].

Because of increasing interest in alternative medicine and anecdotal reports of the beneficial effect of Korean herbal medicine to treat erectile dysfunction, several herbal medicines such as traditional Korean, Chinese[5] and Japanese medicines[6], are being focussed on its availabilities.

Some traditional Korean prescriptions employed for a syndrome expressed in the oriental medical concept as chest paralysis and heartache are thought to be effective for angina pectoris. Therefore, we investigated the effects of an oriental medicinal prescription, Geiji-Bokryung-Hwan (GBH) that is comprised of five herbs of *Cinnamomi Ramulus* (Geiji), *Poria Cocos* (Bokryun), *Mountan Cortex Radicis* (Mokdanpi), *Paeoniae*

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Radix (Jakyak), and *Persicae Semen* (Doin) on anti-atherosclerosis activity. GBH, a well known herbal medicine used in the treatment of cardiovascular disorders (referred to as blood stasis in traditional Korean medicine), which is widely used in China, Japan, Taiwan and Korea. Nonpolar extracts contain some antioxidative substances, which can inhibit platelet aggregation[7] and protect the myocardium against ischemia-induced derangement. An aqueous extract of GBH contains phenolic compounds that are effective in protecting liver microsomes, hepatocytes, and erythrocytes against oxidative damage. For example, a potent hepatoprotective agent and water-soluble antioxidant are suggested[8]. It has previously been reported that the GBH acts as an antioxidant and anti-mutagen and to stimulate phase II drug-metabolizing enzymes (anti-initiation activity); it mediates anti-inflammatory effects and inhibits cyclooxygenase and hydroperoxidase functions (anti-promotion activity); and it induces human promyelocytic leukemia cell differentiation (antiprogression activity). The inhibitory effect of GBH water-extracts on the growth of cancer cell lines such as HepG2 cell and Hep3B cell has been demonstrated. These data suggest that GBH extracts merit investigation as a potential cancer chemopreventive agent in humans, especially in hepatological cancers[9].

Basic fibroblastic growth factor (bFGF) is a family member of the cell differentiating and growth promoting factors. It exists as multiple isoforms ranging from 18 to 24 kD[10]. At the cellular level bFGF is a potent mitogen which promotes cell survival by inhibiting apoptosis, and at the tissue level it can induce angiogenesis and is involved in wound repair[11]. Hypercholesterolemia has been shown to suppress bFGF expression[12]. Caveolin is a 21 to 24 kDa protein and a major component of the caveolar membrane. It is able to interact directly with lipid modified signaling molecules and free cholesterol[13]. Fielding and Fielding[14] reported that in human fibroblasts the cellular free cholesterol level regulates transcription of the gene that encodes caveolin. Also, cholesterol transport to the cell surface was 4 times more rapid in cells expressing caveolin than in matched cells lacking caveolin[15]. Therefore, in addition to functional evaluation, we also obtained penile tissues for morphological studies and to identify protein expression of endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), bFGF and caveolin-1 to determine the mechanism of GBH action.

Materials and Methods

1. Animals

In this study, 3-month-old Sprague-Dawley rats (n=24) were used. The 6 control animals were fed a normal diet and the remaining 18 rats were fed a 1% cholesterol diet for 3 months. After 1 months 30 ㎎/㎏ (group 1) and 60 ㎎/㎏ (group 2). Geiji-Bokryung-Hwan (GBH) were added daily to the drinking water of 6 rats each but not to the remaining 6 rats in the treatment group (cholesterol only group). The ingredients of GBH are *Cinnamomi Ramulus* (Geiji), *Poria Cocos* (Bokryun), *Mountan Cortex Radicis* (Mokdanpi), *Paeoniae Radix* (Jakyak), and *Persicae Semen* (Doin). Serum cholesterol levels were measured at 1 and 3 months. At 3 months erectile function was evaluated with cavernous nerve electrostimulation in all animals. Penile tissues were collected for electron microscopy and to perform Western blot for endothelial and neuronal nitric oxide synthase, bFGF and caveolin-1.

GBH, a prescription formulation, was obtained from Dongguk University Oriental Medical Hospital, Kyungju, Korea (Scheme 1). In addition, for laboratory study, authentic plant materials were purchased from a local market and identified at the Oriental Medical Department, Dongguk University, Kyungju, Voucher specimens [OM-G1, OM-G2, OM-G3, OM-G4 and OM-G5] are on deposit at the Herbarium of Botany Department, Dongguk University, Kyungju, Korea.

Scheme 1. Composition of Geiji-Bokryung-Hwan

Medicinal herb name	Dose amount (g)
Cinnamomi Ramulus	1.33
Poria Cocos	1.33
Moutan Cortex Radicis	1.33
Paeoniae Radix	1.33
Persicae Semen	1.33
Total	6.65

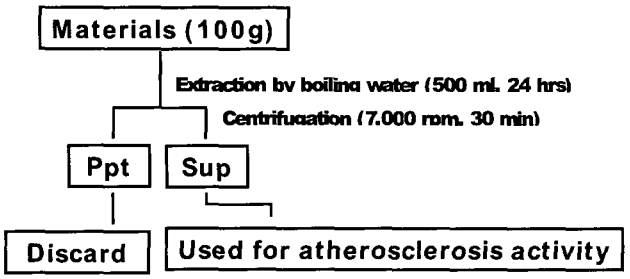
2. Water-Extraction of GBH

The dried plants of GBH were extracted with water (Scheme 2). Plant was cut into small sections, boiled twice in water for 30 mins. The residues were removed by filtration and the combined filtrate was evaporated to obtain the desired concentration (2g dry plant equivalent extract per ml). In a typical experiment, the plant was cut into pieces and macerated in distilled water. The liquid was decanted on day 4 and filtered. The filtrate was evaporated to dryness in an oven at 40℃. The dried extract was then weighed (yield, 1.5%).

3. Electrostimulation

After the rats were anesthetized with 60㎎/㎏ pentobarbital intraperitoneally, a midline abdominal incision was made to enter the pelvis and expose the cavernous nerve. Electrostimulation was performed with a delicate stainless steel bipolar hook electrode attached to a multijointed clamp (each

pole was 0.2㎜. in diameter and the 2 poles were separated by 1㎜). Monophasic rectangular voltage pulses were generated by a computer and converted to current pulses using a custom-built converter. Stimulus parameters were 1.5 mA, frequency 20 Hz, pulse with 0.2 milliseconds and duration 50 secs. The cavernous nerve was stimulated on each side. To monitor intracavernous pressure, the skin overlying the penis was increased and penile crura were exposed by removing part of the overlying ischiocavernous muscle. A 23 gauge needle was inserted into the right crus and connected to a pressure monitor by a polyethylene-50 tubing. Arterial blood pressure was continuously monitored from a catheter (polyethylene-50) inserted into the internal carotid artery. Intracavernous and blood pressures were measured and recorded with a computer using commercial software.



Scheme 2. Preparation of the water-extracts

4. Electron microscopy

Penile samples were immersion fixed in 2% glutaraldehyde and 0.2 M sodium phosphate buffer, pH 7.4, overnight. After fixation in 2% aqueous osmium tetroxide for 2 hours, the tissues were dehydrated in graded (50%, 70%, 90%, 95%, 100%) ethanol and propylene oxide, and subsequently embedded in Epon 812. Thick sections were cut on a microtome, stained with 1% methylene blue and examined for the best structural preservation with light microscopy. Thin sections (approximately 900 A) were mounted on 200 mesh copper grids and stained with 10% uranyl acetate and lead citrate as contrasting agents. Ultrastructural examination was performed with a transmission electron microscope. At × 20,000 the number of caveolae was counted in 4 random areas (1-inch square) of the endothelium and the smooth muscle cells separately. Mean caveolae counts were used for statistical analysis.

5. Western blot

Freshly obtained penile tissues were homogenized on ice in protein lysis buffer containing phosphate buffered saline, pH 7.4, 1% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 10 ㎍/㎖ aprotinin and 10 ㎍/㎖ leupeptin. Insoluble materials were removed by centrifugation (20 min, 14,000 × g, 4℃). Protein concentration was determined using protein assay reagents according to manufacturer protocol. Twenty g from each sample were diluted 1:1 v/v with a 2× sample buffer consisting of 0.125 M tris hydrochloric acid, pH 6.8, 4% SDS, 10% 2-mercaptoethanol, 20% glycerol and 0.004% bromophenol blue. Human foreskin fibroblast, human endothelial, rat pituitary and HeLa cells provided by the manufacturer of the antibodies were also run on the gels as positive controls for caveolin-1, endothelial nitric oxide synthase, neuronal nitric oxide synthase and bFGF, respectively. Samples were then boiled for 3 minutes and loaded onto 15% SDS polyacrylamide gels.

Following electrophoresis proteins were transferred to polyvinylidene difluoride membranes using a semidry transfer unit according to manufacturer protocol. The membranes were stained with a 0.1% ponceau solution to verify integrity of the transferred proteins and monitor the unbiased transfer of all protein samples. The membranes were blocked in tris buffered saline-T (10 mM tris hydrochloric acid, pH 8.0, 150 mM sodium chloride and 0.05% Tween 20 [TBS-T]) plus 5% nonfat dry milk (5% Blotto) overnight at 4℃ and incubated in a dilution of 1:2,000 mouse anti-caveolin-1, 1:1,000 anti-endothelial nitric oxide synthase, anti-neuronal nitric oxide synthase and anti-bFGF in 5% Blotto for 1 hour at room temperature. Membranes were washed 4 times for 10 min in TBS-T and incubated in a 1:15,000 dilution of goat anti-mouse horseradish peroxidase conjugated secondary antibody in 5% Blotto for 1 hour at room temperature. Membranes were then washed 4 times for 10 minutes in TBS-T, and Western blots were developed using an enhanced chemiluminescent kit. Data were compared using the parametric analysis of variance and Mann-Whitney U test with $p < 0.05$ considered significant. Data are expressed as mean plus or minus standard deviation (SD).

Results

1. Effects of Geiji-Bokryung-Hwan on Serum cholesterol levels, systolic blood pressure and peak sustained intracavernous pressure

At months 1 and 3 blood levels of cholesterol were significantly higher in all 1% cholesterol fed groups than in the control group. Furthermore, significant difference was noted among group 1, group 2 and the cholesterol only group. The cholesterol level was greatly reduced after 1 and 3 months of GBH therapy in groups 1 and 2, and systolic arterial pressure was similar in all 4 groups.

Peak sustained intracavernous pressure was significantly lower (48×12 cm. H_2O) in the cholesterol only group, which

was consistent with the results of our pilot study. Treatment with GBH seemed to have a protective effect on erectile function as evidenced by the similar erectile responses in groups 1 and 2 and the controls (Table 1).

Table 1. Serum cholesterol levels, systolic blood pressure and peak sustained intracavernous pressure

Groups	Mean±SD			
	control	group1	group2	cholesterol group
No	6	6	6	6
SC 1	67±11**	122±14	90±12	131±13
SC 3	70±10**	116±15*	122±16*	154±18
BP	103±9	109±8	105±5	111±12
IP	109±15	97±15	103±13	48±12#

SC 1 : Serum total cholesterol(mg/dl) 1 month, SC 3 : Serum total cholesterol(mg/dl) 3 month, BP : Blood pressure 3 months (mmH), IP : Intravernous pressure 3 months (cm H_2O) *, ** : Compared to other groups P<0.05 and P<0.01, respectively. # : Compared to control group P<0.05.

2. Effects of Geiji-Bokryung-Hwan on the number of caveolae in the smooth muscle and endothelial cells of the corpus cavernosum

In smooth muscle and endothelial cells of the control group, and groups 1 and 2 a significantly higher number of caveolae with fingerlike processes in the plasma membrane were noted. In the cholesterol only group smooth muscle cells were mostly round, the number of caveolae was significantly less, and many vesicles were seen near the endoplasmic reticulum and Golgi cisternae (not shown). Of the 6 cholesterol only rats mitochondria accumulation was noted in 5 in which the endothelial cells also had an increased number of vacuoles and a decreased number of caveolae in the cytoplasm (Fig. 1. and Table 2.).

Table 2. The number of caveolae in the smooth muscle and endothelial cells of the corpus cavernosum

	Control	Group 1	Group 2	Cholesterol group (Mean±SD)
No	6	6	6	6
SMC	10.5±1.3	14.7±2.6*	13.3±2.6*	10.1±1.6
EC	20.3±1.8	25.7±3.6*	26.4±3.1*	22.2±3.5

SMC : Smooth Muscle cells, EC : Endothelia cells, * Compared to control group P<0.05

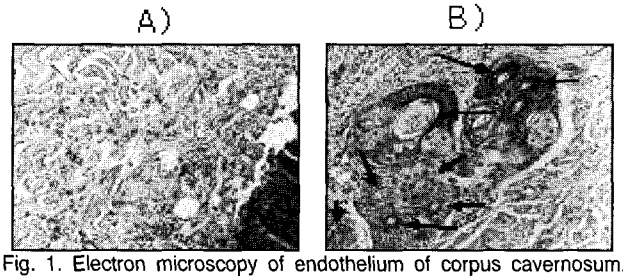


Fig. 1. Electron microscopy of endothelium of corpus cavernosum. A) Geiji-Bokryung-Hwan treatment group B) Vacuoles in endothelium of cholesterol only group (arrows). Reduced from × 20,000 magnitute.

3. Effects of Geiji-Bokryung-Hwan on Caveolin-1 and bFGF protein expression

No difference was seen in protein expression for endothelial and neuronal nitric oxide synthases among the 4 groups. Caveolin-1 and bFGF protein expression was significantly higher in groups 1 and 2 compared to the cholesterol only and control groups. The cholesterol only and control groups had similar levels of protein expression for caveolin-1 and bFGF (Fig. 2).

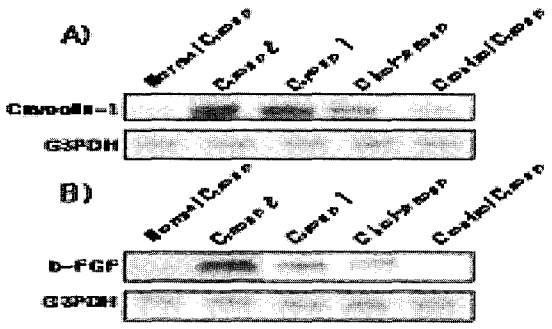


Fig. 2. Western blot for caveolin-1 and bFGF protein. Slight increase of protein expression was noted in cholesterol only group (Chol-Group) compared to control group. GBH treatment groups (Group 1, 30 mg/kg and Group 2, 60 mg/kg) had highest protein expression. Young 3 months-old rats were used as normal group to compare with the Control group. Glyceraldehydes-3-phosphate dehydrogenase (G3PDH) was used as a internal control.

4. Effects of Geiji-Bokryung-Hwan on eNOS and nNOS protein expressions

Western blot showed differences among groups in protein expression for eNOS and nNOS in penile tissue. Increased eNOS and nNOS protein expressions by high cholesterol diet were significantly decreased in group 1 and group 2. Levels of eNOS and nNOS expression were significantly reduced in the treated group.

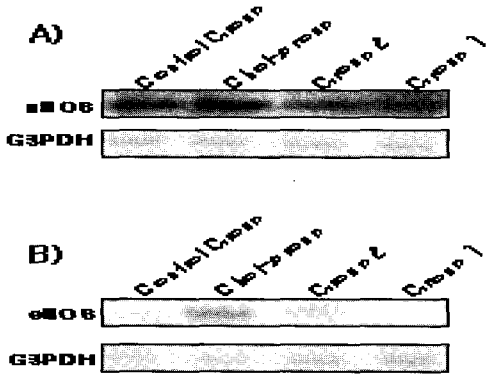


Fig. 3. Western blot for endothelial nitric oxide synthase and neuronal nitric oxide synthase proteins. Significant increase of eNOS protein expression was noted in cholesterol only group (Chol-Group) compared to control group. GBH treated groups (Group 1, 30 mg/kg and Group 2, 60 mg/kg) decreased the protein expression. In contrast, nNOS protein was constitutively expressed in control group and more highly expressed in cholesterol only group (Chol-Group). Korean herbal medicine mixture treated groups (Group 1, 30 mg/kg and Group 2, 60 mg/kg) decreased the protein expression. Glyceraldehydes-3-phosphate dehydrogenase (G3PDH) was used as a internal control.

Discussion

Although vasculogenic erectile dysfunction in an atherosclerotic rabbit model has been extensively studied[3,4]. we investigated the feasibility of a smaller animal model of hypercholesterolemic erectile dysfunction. The rat model offers several advantages. The cavernous nerve can easily be identified and stimulated to assess erectile response. Also, rats are less likely than rabbits to develop wound infection and anesthesia related complications. This study confirms our previous observation that rats consistently develop erectile dysfunction after 3 months of a 1% cholesterol diet.

Anecdotal reports of cure or improvement of erectile dysfunction in men taking traditional Korean herbal medicine prompted us to investigate whether the beneficial effect occurs and whether there is a scientific basis for the cure. Among the many herbal medicine preparations we chose one that is available in health food stores, and the manufacturer was willing to donate the liquid form for our study. We do not know why the preparation contains 5 ingredients. Although pharmacology of individual herbs can be found in traditional Chinese and Korean medicine books, we do not know the pharmacological action of the GBH. Our study indicates that 1 months of GBH seem to protect rats from the harmful effect of hypercholesterolemia and preserve erectile function. The beneficial effect on erectile function was due to reduction of serum cholesterol by herbal medicine as evidenced by similar cholesterol levels among groups 1 and 2, and the cholesterol only group.

No obvious difference in histology was noted among the control, cholesterol only and treatment groups. We hypothesize that the changes may be too subtle to detect under light microscopy and, thus, performed the transmission electron microscopic study. The most interesting change is the significant increase in membrane caveolae in the herbal medicine treated groups. Caveolae are small bulb shaped invaginations at near the cell surface, which can sequester membrane bound ligands away from the extracellular space and facilitate their delivery to the cell cytoplasm. Thyberg et al reported that arterial smooth muscles could shift from a contractile to a synthetic phenotype in vivo and in vitro, and showed that contractile cells, which contain numerous caveolae, do not accumulate lipids in the cytoplasm[16]. They further suggested that contractile smooth muscle cells use caveolin and caveolae to free themselves of excess lipoprotein derived cholesterol, and maintain the balance of intracellular and extracellular cholesterol levels. In an in vitro study of human fibroblast, Fielding et. al, showed up-regulation of caveolin messenger RNA levels by free cholesterol[17]. We noted a small increase of caveolin-1 protein in our cholesterol only group but a large increase in the treated groups. A substantial increase of caveolae and caveolin-1 may compensate or overcome the harmful effect of hypercholesterolemia on the smooth muscle and endothelial cells and, thus, reverse erectile dysfunction in these groups. Further investigation is required to determine how GBH treatment increases the number of membrane caveolae.

The content of GBH in the high cholesterol diet was significantly decreased. It was reported that GBH contains 14.5% antioxidative substances and GBH exerts radical-scavenging activities effectively in the 1,1-diphenyl-2-picrylhydrazyl system[18,19]. It is interesting to note that the relative potency of an antioxidative substance to GBH remained unchanged in the assay systems. This provides indirect evidence that indicates that the origin of the antioxidant activity of GBH was, in fact, predominantly an antioxidative substance. A linear correlation between atherosclerotic area and cholesterol deposition was found. Cholesterol exposure is a known risk factor of atherosclerosis in cholesterol-fed rabbits[20]. Endothelial dysfunction is an early event in the pathogenesis of atherogenesis[21]. The present study demonstrates that GBH treatment significantly reduces endothelial damage.

It has been reported that hypercholesterolemia impairs endothelium mediated relaxation of rabbit corpus cavernosum smooth muscle and, thus, contributes to erectile dysfunction[3]. Chen et. al, also showed that hypercholesterolemia and oxidized low density lipoprotein inhibit angiogenesis and endothelial growth by suppressing endogenous bFGF[12]. They suggested that inducing bFGF expression at targeted sites may improve collateral growth in hyperlipidemic arterial disease. Meurice et. al, further demonstrated that the angiogenic factor bFGF improves endothelial mediated responses in hypercholesterolemic animal models[22]. In our Western blots, bFGF was highly expressed in the treated groups compared to the cholesterol only and control groups. This finding suggests that a mechanism of GBH may be up-regulation of bFGF, which reverses the suppressive effect of hypercholesterolemia on the smooth muscle and endothelium. It is likely that other factors may also be involved and further larger scale studies are needed to determine the mechanisms of action of GBH.

Western blot showed differences among groups in protein expression for eNOS and nNOS protein expression in penile tissue. Increased eNOS and nNOS protein expressions by high cholesterol diet were significantly decreased in group 1 and group 2. Although the prised mechanism of the herbal medicine is unknown, it can be mentioned that high levels of

bFGF and caveolin-1 expression, and decreased levels of eNOS and nNOS expression in the treated group may protect the cavernous smooth muscle and endothelial cells from the harmful effect of high serum cholesterol.

Conclusions

In this pilot study we have confirmed our preliminary observation that rats develop erectile dysfunction consistently after being fed a 1% cholesterol diet for 3 months. Erectile dysfunction was prevented in rats treated with GBH. An increase in membrane caveolae, caveolin-1 and bFGF occurred in the penile tissue of the GBH treated group. In contrast, eNOS and nNOS protein expressions were increased by high cholesterol diet and they were significantly decreased in group 1 and group 2. We hypothesize that these factors may contribute to the beneficial effect of the GBH. Further studies are needed to examine the effect of GBH on other organ systems and determine its mechanisms of action.

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References

1. Ross R. The pathogenesis of atherosclerosis: a perspective for 1990s. Nature 362, 801-809, 1993.
2. Wei, M., Macera, C.A., Davis, D.R. et al: Total cholesterol and high density lipoprotein cholesterol as important predictors of erectile dysfunction. Am J Epidemiol, 140:930, 1994.
3. Kim, J.H., Klyachkin, M.L., Svendsen, E. et al: Experimental hypercholesterolemia in rabbits induces cavernosal artherosclerosis with endothelial and smooth muscle cell dysfunction. J Urol, 151:198, 1994.
4. Azadzoi, K.M. and Saenz de Tejada, I.: Hypercholesterolemia impairs endothelium-dependent relaxation of rabbit corpus cavernosum smooth muscle. J Urol, 146:238, 1991.
5. Bakircioglu, M.E., Hsu, K., El-Sakka, A., Sievert, K.D., Lin, C.S., Lue, T.F. Effect of a Korean herbal medicine on a rat model of hypercholesterolemic erectile dysfunction. J. Urology 164, 1798-1801, 2000.
6. Yoshie, F., Iizuka, A., Kubo, M., Komatsu, Y., Matsumoto, A., Itakura, H., Takeda, H., Matsumiya, T., Kondo, K. Protective effects of Saiko-ka-ryukotsu-borei-to (Chai-Hu-Jia-Long-Gu-Mu-Li-Tang) against atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits. Pharmacol Res. 43(5):481-488, 2001.
7. Kim, J.G., Kim, C.H., Park, S.Y., Kim, J.S., Choi, J.Y., Kim, C.H., Park, W.H. An experimental study on the effect of Geijibokryunghwan, Dangguijakyaksan and each constituent herb on inhibition of platelet aggregation. J Kor Inst Ori Med Diagnost 4, 71-84, 2000.
8. Joo, S.T. Effects of the Geiji-Bokryung-Hwan on carrageenan-induced inflammation and COX-2 in hepatoma cells. Thesis of Degree of Master, Dongguk Univ. Graduate School, Kyungju, Korea. 2001.
9. Lee, S.K., Kim, J.G., Kim, C.H., Park, S.Y., Kim, J.S., Choi, J.Y., Kim, C.H., Park, W.H. Effect of the Geijibokryunghwan on human hepatocarcinoma cells. J Kor Inst Ori Med Diagnost In Press. 2002.
10. Estival, A., Monzat, V., Miquel, K. et al Differential regulation of fibroblast growth factor (FGF) receptor-1 mRNA and protein by two molecular forms of basic FGF. Modulation of FGFR-1 mRNA stability. J Biol Chem, 271:5663, 1996.
11. Fox, J.C. and Shanley, J.R.: Antisense inhibition of basic fibroblast growth factor induces apoptosis in vascular smooth muscle cells. J Biol Chem, 271:12578, 1996.
12. Chen, C.H., Cartwright, J., Jr., Li, Z. et al Inhibitory effects of hypercholesterolemia and ox-LDL on angiogenesis-like endothelial growth in rabbit aortic explants. Essential role of basic fibroblast growth factor. Arterioscler Thromb Vasc Biol, 17:1303, 1997.
13. Murata, M., Peranen, J., Schreiner, R. et al VIP21/caveolin is a cholesterol-binding protein. Proc Natl Acad Sci USA, 92:10339, 1995.
14. Fielding, C.J. and Fielding, P.E. : Intracellular cholesterol transport. J Lipid Res, 38:1503, 1997.
15. Smart, E.J., Ying, Y., Donzell, W.C. et al A role for caveolin in transport of cholesterol from endoplasmic reticulum to plasma membrane. J Biol Chem, 271:29427, 1996.
16. Thyberg, J., Calara, F., Dimayuga, P. et al Role of caveolae in cholesterol transport in arterial smooth muscle cells exposed to lipoproteins in vitro and in vivo. Lab Invest, 78:825, 1998.
17. Fielding, C.J., Bist, A. and Fielding, P.E. Caveolin mRNA levels are up-regulated by free cholesterol and down-regulated by oxysterols in fibroblast monolayers. Proc Natl Acad Sci USA, 94:3753, 1997.
18. Park, W.H., Kim, K.S., Kim, K.H., Kim, D.S., Kim, C.H. The antiplatelet activity of Geiji-Bokryung-Hwan, Korean traditional formulation, is mediated through inhibition of phospholipase C and inhibition of TxB(2) synthetase activity. Int. Immunopharmacol. 3(7):971-978, 2003.

19. Kim, B.J., Kim, Y.K., Park, W.H., Ko, J.H., Lee, Y.C., Kim, C.H. A water-extract of the Korean traditional formulation Geiji-Bokryung-Hwan reduces atherosclerosis and hypercholesteremia in cholesterol-fed rabbits. Int. Immunopharmacol. 3(5):723-734, 2003.
20. Sparrow, C.P., Doebber, T.W., Olszewski, J., Wu, M.S., Ventre, J., Stevens, K.A., Chao, Y.S. Low density lipoprotein is protected from oxidation and the progression of atherosclerosis is slowed in cholesterol-fed rabbits by the antioxidant N,N'-diphenyl-phenylene-diamine. J Clin Invest, 89:1885-1891, 1992.
21. Lusis, A.J., Navab, M. Lipoprotein oxidation and gene expression in the artery wall new opportunities for pharmacological intervention in atherosclerosis. Biochem. Pharmacol. 46, 2119-2126, 1993.
22. Meurice, T., Bauters, C., Vallet, B. et al bFGF restores endothelium-dependent responses of hypercholesterolemic rabbit thoracic aorta. Am J Physiol, 272: H613, 1997.