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Ⅱ．過去の研究歴

1992.4～1993.3 富山医科薬科大学和漢薬研究所難波恒雄教授の教室で一年間の研究を終え、名古屋市立大学薬学部荻原幸夫教授の研究室において、漢方方剤の薬効解析についての研究をはじめた。

Ⅲ．過去の研究実績

1. 生薬学雑誌 A Comparative Study of Alkaloidal Constituents in Corydalis decumbens Tubers by HPLC　47(4) 440-445 1993

2. 第2回日本動力脈硬化学会総会 1995.3.30-31 漢方処方の解析(第7報) 黄芩拮抗甘麦湯加参の抗高脂血作用

3. 第76回日本東洋医学会関西支部例会 1996.11.17 〃 黄芩拮抗甘麦湯加参の抗高脂血作用およびその作用機序に関する

Ⅳ．本年度の研究業績

(1) 学会、研究会等においての口頭発表（学会名・内容）

International symposium on Natural Medicines. in kyoto 10.28-30 1997
EVALUATION OF KAMPO-SHOHO (No. 100)
ANTICHOLESTERMIC ACTION OF KAMPO MEDICINE OGI-KEISHI-GOMOTSU-TO-KA-KOTIN (OKGK)

(2) 学会誌等に発表した論文　　無 ・ (有)　（雑誌名・論文名）

① Phytomedicine　4(4) 295-300 1997
Antihyperlipidimic Action of a trandition'al chinese Medicine (Kampo medicine) Ogi-Keishi-Gomotsu-To-Ka-Kojin

② Biol. pharm. Bull submitted Antihypercholesteremic action of a Kampo Medicine. OKGK.

Ⅴ．今後の研究計画及び希望

黄芩拮抗甘麦湯加参の有効成分を分画分離により探索する。有効成分の作用メカニズムを方剤のそれと比較しながら検討する。より有効な動力脈硬化治療薬の開発を可能にする。そして、漢方研究の有用性、重要性を示したい。

Ⅵ. 研　究　報　告（日本語、又は英語で書いて下さい。 4,000字以上で記載して下さい。別紙可）

別紙

Ⅶ. 指導教官の意見

本人は笹川医学奨学金制度研修生として来日，帰国後急学の念止みがたく，本生薬学教室の大学院生として進学した。以後4年着々と成果を挙げ、明年3月には博士の学位を取得可能となった。非常に熱心に実験に取組んでおり，漢方薬の科学的評価という地味な分野ではあるが、注目に値する研究者として成長している。本助成によりこの一年は経済的にもゆとりが出て実験が進んでいる。指導者としても財団に感謝を表する次第である。

研究報告書

呉　春珍

近年、喫煙、高血圧、高脂血症および糖尿病など心血管系疾患の主たる危険因子の発見によって、その予防、治療に大きな進歩が遂げられてきた。これによって心血管系疾患発症率を有意に低下させた国も少なくない。しかし、このように大きな成果が得られているにもかかわらず、心血管系疾患はいまだに米国など多くの先進工業国において死因のトップになっている。日本人の食生活の欧米化によって、肥満、高脂血症など代謝異常が大幅に増加した。高脂血症、特に高コレステロール(CHO)血症と動脈硬化疾患の一つである虚血性心疾患との関連性については、広く研究され、高CHO血症がその発症や病態進行の危険因子であることが報告されている。高脂血症は血管壁に沈着するコレステロールを供給することにより、また、血管壁の性状、血球の性質に影響をおよぼすことにより、動脈硬化発症に関与している。また、1%の血漿コレステロール値の低下は冠動脈硬化症を2%抑制することが報告されており、高コレステロール血症の治療は粥状動脈硬化症治療にとっては不可欠な治療と考えられる。

一方、血清トリグリセライド（TG）値の上昇と心血管系疾患のリスク増大との間に相関関係があることが多くの研究から明らかにされており、高TG血症は閉経後の女性の動脈硬化症の危険因子とされている。高TG血症では、高比重リポ蛋白(HDL)コレステロールの低下を伴うことが多く、高レムナント血症を生じて血管壁のコレステロール蓄積を促進し、また、血栓形成を助長することにより血管壁の病的反応を引き起こして冠動脈硬化症を進展させると考えられる。血清CHOのみならず血清TGを低下させることは心血管系疾患の予防および治療において重要な課題と考えられる。

古来より、主として東洋で使われてきた漢方方剤は、合成薬品に比べて比較的副作用が少なく、予防を目的とする長期投与が可能であるという特長がある。本研究室では、いくつかの漢方方剤が抗高脂血症作用、あるいは、抗動脈硬化作用を示すことを明らかにしてきた。循環器系疾患のように多くの因子が複雑に絡み合って発症する慢性疾患には、長期間使用可能な漢方方剤の様な薬剤が有用であると考えられる。また、漢方方剤の抗高脂血症作用、抗動脈硬化作用を検討することは、漢方方剤の作用機序を明らかにし、臨床上の有用性を裏付けるというだけでなく、新たな抗高脂血症作用、抗動脈硬化作用の機序が見いだされる可能性があり、新規薬剤の開発にもつながる可能性を秘めている。

黄耆桂枝五物湯加紅参は金匱要略の血痺虚労症脈症弁治に見られる処方で、これまでに末梢神経系、循環器系の異常によると考えられるスモン患者の手足のしびれ、冷え、さらに運動機能障害の改善に有効であることが臨床的に示されてきた。しかし、これまでに、黄耆桂枝五物湯加紅参の作用機序に関する基礎的な研究は殆どない。そこで、本研究では、本方剤の循環器系疾患の改善効果を有する可能性を検討するために、抗高脂血症作用に焦点を絞り、ラットを用いた高コレステロール血症モデル、高TG血症モデルを作成して、本方剤の血清と肝臓のTG、CHO、およびリン脂質(PL)低下作用、および、その作用機序について検討した。

脂質代謝異常が直接因子として動脈硬化症の成立に重要な役割を演じることは異論のないところである。脂質代謝異常の中でも高CHO血症は動脈硬化症の発症因子として重要視されており、また、高TG血症は高CHO血症に比べ虚心性心疾患の発症率と相関が高いことが報告されている。現在、ヒトの脂質代謝異常を反映する動物モデルはないと考えられているが、実験的高脂血症動物は脂質代謝のしくみを知り、ヒトの高脂血症の病態を解明する手段として重要である。本研究では、脂質代謝異常を高CHO血症、高TG血症に絞り、血清CHOを増加させるコレステロール食を用い、長期間負荷することにより作成した高脂血症ラットモデルに対する本方剤の効果を調べた。

一方、Yudkinら が疫学的調査からショ糖摂取量と虚血性心疾患の発症率との間に正の相関のあることを報告して以来、ショ糖およびその構成成分であるフルクトースによる脂質代謝異常が注目されるようになった。そこで、今回、12週間グリセロール/フルクトース負荷による高TG血症ラットモデルを作成し、高TG血症ラットに対する本方剤の効果を調べた。本研究の結果は次のように要約される。

1、抗高CHO血症作用とその作用機序についての検討

6週齢雄性SDラットを1%コレステロール含有飼料で、12週間飼育することにより、高コレステロール血症ラットを作成した。黄耆桂枝五物湯加紅参はヒト常用量の2倍あるいは10倍に相当する量をラットの餌一日摂取量中に含有するよう調製して与えた。すでに、黄耆桂枝五物湯加紅参はラットの高脂血症モデルにおいて、抗高コレステロール血症と同時に、強力な抗高トリグリセリド血症作用を見い出しており、血清Ｔ－ＣＨＯ低下作用機序についてラット初代培養肝細胞を使用して検討したところ、CHO合成の抑制が観察されたが、HMG-CoAレダクターゼには活性低下は見られず、原因酵素の同定はできなかった。一方、腸管からのコレステロール吸収抑制作用が観察され、高コレステロール食負荷時には肝臓での内因性コレステロール合成は通常より低下することを考えると、本方剤の血清T-CHO低下作用は腸管からのCHO吸収抑制が主因になると考えられる。さらに、今回コレステロールから胆汁酸を合成する段階の律速酵素であるコレステロール７〆ーヒドロキシラーゼの活性に対する本方剤の影響を検討した、コレステロール７〆ーヒドロキシラーゼの活性の増加作用が観察された。この結果から、血清Ｔ－ＣＨＯ低下作用は、コレステロールの胆汁酸としての排泄促進が関与することが示唆された。

2、抗高TG血症作用とその作用機序についての検討

6週齢雄性SDラットに15%グリセロール/15%フルクトース(GF)含有水を12週間自由摂取させることにより、高TG血症ラットを作成した。方剤投与群では、GF含有水を与えながら黄耆桂枝五物湯加紅参をヒト常用量の5倍あるいは10倍量を経口投与した。黄耆桂枝五物湯加紅参ヒト常用量の5倍量群と10倍量群のいずれでも血清TG及びPLの増加が3ケ月間の同時投与によって、有意に抑制されることが認められた。血清TGおよびPL低下作用機序についてラットを用いて検討したところ、本方剤は14C酢酸と3Hオレイン酸の肝臓TG画分中への放射活性の取り込みを抑制し、また、肝臓ミクロソーム中のTG合成酵素のDGAT活性に対する抑制を示した。さらに、リポ蛋白のTG異化代謝に重要な役割を果たしているHTGL, LPLの活性を増加させた。本実験に用いたフルクトース負荷による高TG血症の成因は、肝におけるTG合成の亢進と肝からのTG分泌亢進およびリポ蛋白リパーゼの活性低下に起因する血中TGの分解抑制によると考えられている。また、グリセロール負荷による高TG血症の発症機構は異論はあるが、肝におけるTG合成亢進あるいは、内因性TGの除去障害によると考えられている。すなわち、黄耆桂枝五物湯加紅参は、肝臓でのTG合成を抑制することが血清TG低下の一因になると考えられる。また、リポ蛋白リパーゼ活性と肝性リパーゼ活性を増加させることにより、内因性TGの加水分解を促進することが、血清TG低下のもう一つ重要な原因になると考えられる。

黄耆桂枝五物湯加紅参は黄耆桂枝五物湯と紅参から構成された処方であり、今回さらに、この二つの構成成分との抗高TG血症作用を比較した。高TG血症モデルに対して、黄耆桂枝五物湯加紅参、黄耆桂枝五物湯と紅参はともに血清TG低下作用が観察された、強さは黄耆桂枝五物湯加紅参、紅参、黄耆桂枝五物湯の順でした。また、HTGL, LPLの活性を増加させた、黄耆桂枝五物湯加紅参は黄耆桂枝五物湯、紅参単独より強かった。

一方、コレステロール７〆ーヒドロキシラーゼの発現組織はおもに肝臓であり、LPLの発現組織は脂肪組織、心臓、骨格筋などに分布している。そこで、肝臓、脂肪組織、心臓、ヒラメ筋からｔｏｔａｌＲＮＡを抽出し、コレステロール７〆ーヒドロキシラーゼおよびLPLのｍＲＮＡを測定した、黄耆桂枝五物湯加紅参の投与によるｍＲＮＡレベルの変化は見られなかった。酵素活性の増加作用は転写後の調節によると考えた。酵素活性の調節機序についてはさらに検討する必要があると考える。

1P-038

EVALUATION OF KAMPO-SHOHO (No. 100) ANTICHOLESTEREMIC ACTION OF KAMPO MEDICINE OGI-KEISHI-GOMOTSU-TO-KA-KOJIN(OKGK)

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Ogi-keishi-gomotsu-to-ka-kojin(OKGK)[1] is a Kampo medicine that is composed of six medicinal plants and has been used for improvement of the sensory disorder of limbs derived from the malfunction of nervous system. In this study we undertook to examine the effect of OKGK on cholesterol metabolism in rats.

<METHEDS>

Male SD rats were fed a cholesterol-enriched diet or supplemented with OKGK for 12 weeks. Their serum and liver total cholesterol (TC) were measured every 2 weeks. In order to clarify the mechanism of antihypercholesteremic action, cholesterol absorption was studied using Dual isotope radio methed[2] and cholesterol excretion was determined by measuring of excretion of intravenously injected ^{14}C-cholesterol into feces. Furthermore, liver microsomal cholesterol 7α-hydroxylase activity was measured.

<RESULTS AND CONCLUSIONS>

Effects of OKGK on cholesterol metabolism were examined in experimentally induced rat hypercholesteremic model. The serum and liver total cholesterol (TC) were elevated significantly by feeding of a cholesterol-enrich diet. The supplement of OKGK at 1.25% in diet significantly inhibited the increase of serum TC and liver TC at 1.25%,0.25% in diets. These results suggest that OKGK is effective in the treatment of hypercholesteremia which is induced by exogenous cholesterol and fat enrich-diet. When the mechanism of the anticholesteremic action of OKGK was investigated in detail, OKGK decreased cholesterol absorption in the alimentary tract and also increased the excretion of intravenously injected ^{14}C- cholesterol into feces. Furthermore, OKGK enhanced the activity of cholesterol 7α-hydroxylase in normal and hypercholesteremic rats, but had no effect on HMG-CoA reductase activity. These results suggest that the anticholesteremic action of OKGK are in part due to the inhibition of cholesterol absorption and increase of cholesterol excretion by stimulation of cholesterol 7α-hydroxylase activity .

References

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Antihyperlipidemic action of a Traditional Chinese Medicine (Kampo Medicine), Ogi-Keishi-Gomotsu-To-Ka-Kojin

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Summary

The antihyperlipidemic action of Ogi-Keishi-Gomotsu-To-Ka-Kojin (OKGK) was examined in rats with experimentally-induced hyperlipidemia. Oral administration of OKGK at 0.69 or 1.38 g/kg/day significantly reduced the increase of serum triglycerides (TG) and phospholipids (PL). OKGK given as a nutritional supplement (1.25%) in the diet significantly suppressed the increase of serum TG and PL in rats with hypercholesterolemia induced by a cholesterol and fat-enriched diet. These results suggest the effectiveness of OKGK in the treatment of hypertriglyceridemia induced by both endogenous and exogenous TG. The mechanism by which OKGK shows antihypertriglyceridemic action was investigated in detail. OKGK decreased [^{14}C] acetic acid incorporation into TG and PL but not fatty acids in primary cultured rat hepatocytes from rats treated with oral OKGK for 1 week. OKGK also reduced [^{3}H] oleic acid into TG and PL, suggesting OKGK suppresses TG and PL syntheses in the liver. Furthermore, OKGK enhanced the activities of lipoprotein lipase (LPL) and hepatic TG lipase (HTGL) in postheparin plasma. These results suggest OKGK inhibits triglyceride synthesis in the liver and stimulates the hydrolysis of TG in lipoprotein.

Key words: Kampo medicine; hypertriglyceridemia; lipoprotein lipase; hepatic triglyceride lipase.

Introduction

Hypercholesterolemia is directly linked to the initiation and progression of atherosclerosis. Considerable evidence indicates that oxidatively modified low density lipoprotein (LDL) induces macrophage-derived foam cells, resulting in fatty streak formation, which is an early characteristic of atherosclerosis. Furthermore, β-very low density lipoprotein (β-VLDL), VLDL and chylomicron remnants are considered atherogenic. On the other hand, there is increasing evidence of the relationship between triglyceride-rich lipoprotein and the progression of coronary heart disease. The Paris prospective study (Fontbonne, 1989) and the Stockholm Heart study (Carlson and Rosenhamer, 1988) also indicated that hypertriglyceridemia is both a risk factor for coronary heart disease in post-menopausal women. Furthermore, hypertriglyceridemia is linked to impaired fibrinolytic function (Hamsten et al., 1985; Nilsson et al., 1985), suggesting that it stimulates clot formation inducing thrombus and arteriosclerosis. Hypertriglyceridemia is usually accompanied by high VLDL and chylomicrons, due to high dietary fat, a high TG synthesis in the liver and impaired hepatic triglyceride lipase (HTGL) and lipoprotein lipase (LPL) activities. HTGL and LPL are critical enzymes in the

Abbreviations OKGK – Ogi-Keishi-Gomotsu-To-Ka-Kojin; LPL – lipoprotein lipase; HTGL – hepatic triglyceride lipase; TG – triglyceride; PL – phospholipid; TC – total cholesterol; FA – fatty acid; LDL – low density lipoprotein; VLDL – very low density lipoprotein; IDL – intermediate density lipoprotein; HDL – high density lipoprotein; SD – Sprague-Dawley; PBS – phosphate-buffered saline; BSA – bovine serum albumin; GF water – 15% glycerol and 15% fructose-containing water

regulation of lipoprotein and lipid metabolism. Recent studies using transgenic mice indicated that overexpression of LPL stimulates hydrolysis of TG in lipoprotein, resulting in the reduction of serum TG (Liu et al., 1994).

On the other hand, overexpression of HTGL in transgenic mice reduced cholesterol accumulation in the aorta during a hyperlipidemic diet (Busch et al., 1994) and that in transgenic rabbits decreased plasma cholesterol concentration and intermediate density lipoprotein (IDL) (Fan et al., 1994). These observations suggest that LPL and HTGL activities are implicated in the protection against atherosclerosis. In this respect, improvement of hypertriglyceridemia in addition to hypercholesterolemia is required to prevent atherosclerosis and arteriosclerosis. Several traditional Chinese medicines (Kampo medicines) exhibit antihyperlipidemic action (Shen et al., 1996). Ogi-Keishi-Gomotsu-To-Ka-Kojin (OKGK) is a Kampo medicine that is composed of six medicinal plants: *Astragali* radix, *Cinnamomi* cortex, *Paeoniae* radix, *Zingiberis* rhizoma, *Zizyphi* fructus, *Ginseng* radix rubra and has been used for improvement of the sensory disorder of the limbs. However, its pharmacological and biochemical effects have not yet been investigated. We therefore focused on the effects on the circulatory system and investigated the effect of OKGK on hyperlipidemia.

Materials and Methods

Animals

Male Sprague-Dawley (SD) rats weighing 170–190 g from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) were maintained under controlled laboratory conditions.

Preparation of OKGK

Crude drug composition of OKGK is described in Table 1. Plant materials used in this study, of which grades were conformed to Japanese pharmacopoeial standards, was authenticated and provided by Tsumura Co. Ltd., Tokyo, Japan. Six crude drugs were added to 700 ml of distilled water, boiled for 1 h using an electric heater and concentrated to 300 ml. This decoction was filtered and lyophilized to give 7.5 g of powdered extract. The main constituents are astraisoflavan, cinnamic aldehyde, paeoniflorin, gingerol and oleanolic acid.

Hyperlipidemia rat model

Rats were randomized into four groups. Group 1 was used as a normal group and group 2 received drinking water containing 15% glycerol and 15% fructose (GF water). Groups 3 and 4 received a single oral administration of OKGK suspended in distilled water at doses of 0.69 and 1.38 g/kg body weight/day for 12 weeks with the aid of a 20 gauge feeding needle, respectively, while receiving simultanously GF water. Diet, water and GF water were given *ad libitum*. To evaluate antihypercholesterolemic effects, rats were randomized into four groups. Group 1 was fed a normal chow diet (CE-2, Nippon Crea Co., Ltd., Shizuoka, Japan). Group 2 was fed a cholesterol and fat-enriched diet, which was CE-2 fortified with 1% cholesterol, 0.2% cholic acid and 2.5% olive oil. Groups 3 and 4 received a cholesterol and fat-enriched diet supplemented with 0.25% and 1.25% OKGK for 12 weeks, respectively. These diets and water were given *ad libitum*.

Determination of serum lipids

Rats were fasted for 16 h and blood samples obtained by cardiac puncture every 2 weeks. Serum was prepared from samples centrifugated at 3000 rpm for 15 min. TG, total cholesterol (TC) and phospholipids (PL) in serum were determined by the cholesterol oxidase method.

Hepatocyte isolation and lipid synthesis

Hepatic parenchymal cells were isolated from male SD rats (300 g) by two-step collagenase perfusion method (Seglen, 1972). Cell viability was determined with 0.25% trypan blue and more than 91.3 ± 1.0 (n = 9) % were used in this study. Hepatocytes were seeded in collagen-coated 12-multi-well culture dish at a density of 10^5 cells/cm^2 with 0.8 ml of William's E medium (pH 7.4) supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin, 10^{-7}M insulin and 10^{-7}M dexamethasone. After incubation for 4 h at 37 °C in an atmosphere of 95% air and 5% CO_2, hormone-containing medium was removed from the wells. The cells were washed twice with phosphate-buffered saline (PBS) and then incubated with

Table 1. Crude drug composition of OKGK

Plant name	Part used	Composition (g)
Astragalus membranaceus Bunge (Lamiaceae)	Root	8
Cinnamomum cassia Blume (Lauraceae)	Bark	4
Paeonia lactiflora Pallas (Paeioniaceae)	Root	4
Zingiber officinale Roscoe (Zingiberaceae)	Rhizome	4
Zizyphus jujuba Miller. var. *inermis* Rehder (Rhamnaceae)	Fruit	4
Panax ginseng C. A. Meyer (Araliaceae)	Root	3

hormone free medium containing [2-^{14}C] acetic acid (0.5 μ Ci/well) and [^{3}H] oleic acid (0.5 μ Ci/well) for 4 h. After the incubation, cells were thoroughly washed twice with PBS and the cellular lipids were extracted 2 times with 2 ml of hexane/isopropanol (3:2, v/v). Fatty acid (FA), TG, and PL were separated by thin layer chromatography using hexane/diethyl ether/acetic acid (50:20:1 v/v) as a solvent system. Radioactivity in regions corresponding to lipid standards was measured by liquid scintillation spectrometry. Protein was determined according to the method of Lowry (Lowry, et al, 1951) using bovine serum albumin (BSA) as a standard and data were normalized to the amount of cellular protein.

Assay of postheparin plasma hepatic lipase (HTGL) and lipoprotein lipase (LPL)

SD rats were treated with OKGK at a dose of 1.38 g/kg for 2 weeks and blood samples were obtained at 9 a.m. Rats were injected i.v. with 20 units of heparin/100 g of body weight and 5 ml of blood was drawn from vein cava inferior 10 min later. Plasma samples were stored immediately at −80 °C. Hepatic lipase (EC 3.1.1.3) activity was assayed as follows. The assay mixture consisted of 1.33 mM [^{3}H] trioleylglycerol (10^{6} dpm/μmol), 0.1 mM lysophosphatidylcholine, 0.2 M Tris-HCl buffer (pH 9.0), 0.4% BSA, 0.5 M NaCl and postheparin plasma in a total volume of 200 μl. The reaction was started by the addition of plasma. After incubation at 30 °C for 10 min with shaking, the reaction was terminated with 3.25 ml of methanol/chloroform/heptane (1.41:1.25:1 v/v), followed by 1.05 ml of 0.1 M borate buffer (pH 10.5) (Nilsson-Ehle and Ekman, 1977). The assay mixture was shaken vigorously with a vortex mixer and the released fatty acid recovered in the upper phase. One ml of the upper phase was taken out to count the radioactivity by scintillation spectrometry.

Lipoprotein lipase (EC 3.1.1.34) in postheparin plasma was assayed as described in the measurement of HTGL activity, except that NaCl was not added in assay mixture and that all assays were carried out at 37 °C. Activity was expressed as a unit which represents nmol of free fatty acids released per hour per ml of plasma.

Chemicals

[2-^{14}C] Acetic acid, sodium salt (3.1 mCi/mmol), [9,10-^{3}H] oleic acid (9.2 Ci/mmol), [9,10-^{3}H] triolein (28.0 Ci/mmol) were purchased from Dupont NEN Research products, Boston, MA. 1,2-dioleyl-sn-glycerol was obtained from Sigma chemical Co, St Louis MO.

Table 2. Effect of OKGK on incorporation of [^{14}C] acetic acid into lipids of rat primary cultured hepatocytes.

		Normal	OKGK	Inhibition	
		(dpm/mg protein)	(dpm/mg protein)	%	Means of three experiments
TG	exp. 1	50 406 ± 426	30 559 ± 602**	39.4	37.8 ± 3.3
	exp. 2	62 554 ± 2727	43 460 ± 1168**	31.5	
	exp. 3	79 647 ± 489	45 791 ± 1677**	42.5	
PL	exp. 1	76 673 ± 2844	65 255 ± 112*	14.9	29.8 ± 7.7
	exp. 2	111 369 ± 1139	73 532 ± 2302**	34.0	
	exp. 3	159 760 ± 507	94 973 ± 8816*	40.6	
FA	exp. 1	426 ± 36	414 ± 35	2.8	2.5 ± 0.9
	exp. 2	490 ± 41	471 ± 44	3.9	
	exp.3	6 486 ± 194	6 434 ± 356	0,9	

Values represent mean ± S.E. of 4 wells. * $p < 0.05$, ** $p < 0.01$ vs. normal group.

Table 3. Effect of OKGK on incorporation of [^{3}H] oleic acid into lipids of rat primary cultured hepatocytes.

		Normal	OKGK	Inhibition	
		(dpm/mg protein)	(dpm/mg protein)	%	Means of three experiments
TG	exp. 1	526 682 ± 3 168	353 173 ± 4576**	32.9	31.7 ± 4.2
	exp. 2	655 964 ± 10 308	508 470 ± 3106**	22.5	
	exp. 3	1 240 423 ± 35 587	785 724 ± 1315**	36.7	
PL	exp. 1	344 244 ± 10 645	245 465 ± 4912**	28.7	25.4 ± 1.9
	exp. 2	373 956 ± 9 385	282 421 ± 3650**	25.5	
	exp. 3	466 980 ± 7 590	364 175 ± 2186**	22.1	

Values represent mean ± S.E. of 4 wells. * $p < 0.05$, ** $p < 0.01$ vs. normal group.

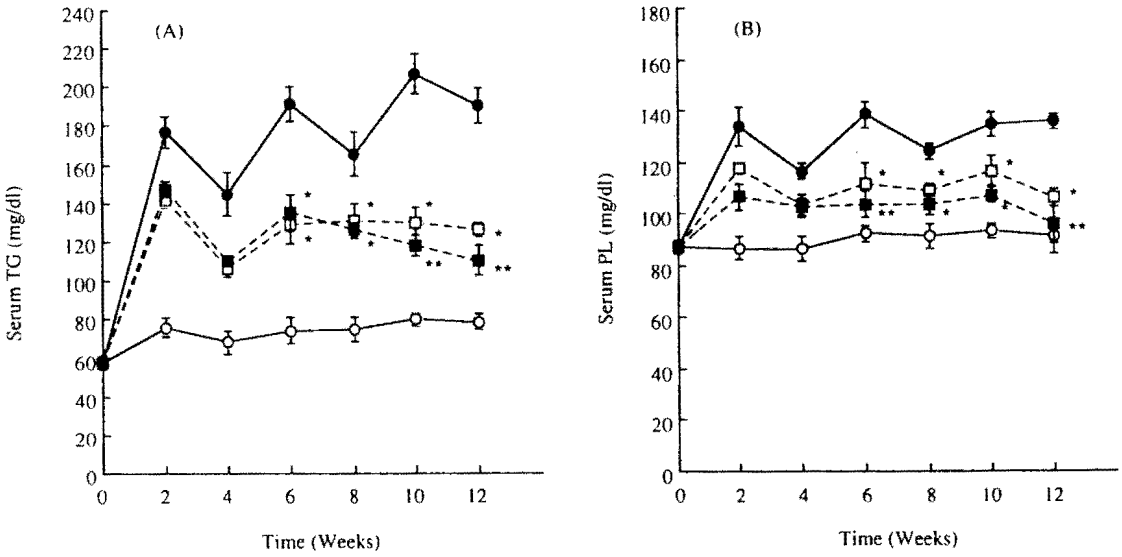

Fig. 1. Effect of OKGK on serum (A) TG and (B) PL in hypertriglyceridemic rats. Sprague Dawley rats were fed a normal chow while giving GF water for 12 weeks. Serum TG and PL were determined every two weeks. O: normal, ●: control (GF water), □: GF water and 0.69 g/kg of OKGK, ■: GF water and 1.38 g/kg of OKGK. Values represent mean ± S.E. of 5 rats. * $p < 0.05$, ** $p < 0.01$ vs. control group.

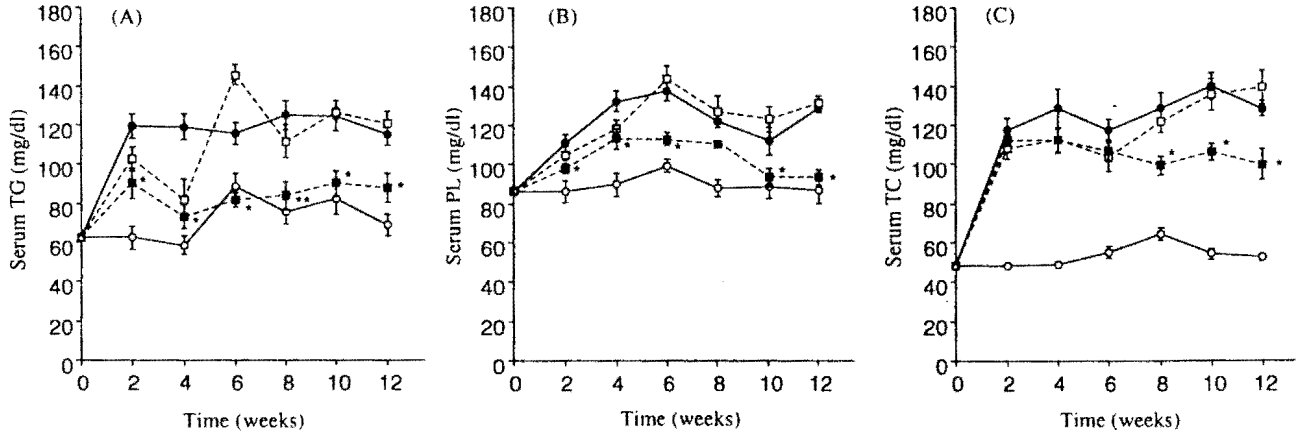

Fig. 2. Effect of OKGK on serum (A) TG, (B) PL and (C) TC in hypercholesterolemic rats. Sprague Dawley rats were fed a cholesterol and fat-enriched diet for 12 weeks. Serum triglyceride, phospholipid and total cholesterol were determined every two weeks. O: normal, ●: control (rats fed a cholesterol-fat enriched diet), □: rats fed a control diet supplimented with 0.25% of OKGK, ■: rats fed a control diet supplimented with 1.25% of OKGK. Values represent mean ± S.E. of 7 rats. * $p < 0.05$, ** $p < 0.01$ vs. control group.

Statistical analysis

Results were represented as mean ± S.E. with the numbers of animals or experiments in parentheses. Statistical significance was determined by the Student's *t*-test. *P* values < 0.05 were considered significant.

Results and Discussion

Antihypertriglyceridemic action of OKGK in hypertriglyceridemia rat model. As shown in Fig. 1, intake of GF water markedly raised the serum TG levels 2–3 fold over normal level during the period of experiment. Serum PL also increased by 30–40 mg/dl concomitantly. OKGK at the doses of 0.69 and 1.38 g/kg, which corresponded to 5 and 10 times of human daily dose, significantly lowered TG and PL levels relative to those in control group. Both doses of OKGK did not change body weight compared with normal or control group. TG accumulation in liver was not observed in this model. Glycerol and fructose are known to augment the production of endogenous TG and OKGK reduced serum TG level in this model, thus suggesting that OKGK would inhibit TG synthesis or stimulate its degradation.

Antihypertriglyceridemic action of OKGK in hypercholesterolemia rat model. We next studied the antihypertriglyceridemic action of OKGK on hypercholesterolemic rats. In this rat model, serum TC increased about 3 fold, serum TG did 1.5–2 fold and serum PL did about 1.5 fold over normal level. OKGK showed a powerful lowering effect on serum TG and PL at a higher dose, whereas such a potent effect was not seen at a lower dose (Fig. 2). No difference in body weight among each group was observed for the entire period. On the other hand, although this model was usually used as a hypercholesterolemia model, the effect of OKGK was not potent and serum cholesterol was slightly reduced at a high dose of OKGK after 8 weeks of starting the administration. Since the increased serum TG in this model was mainly derived from dietary fat, OKGK would presumably suppress the absorption of TG at alimentary tract by inhibiting the degradation of TG by lipase or the resynthesis of TG in epithelial cells besides the suppression of TG synthesis in liver and the stimulation of TG degradation.

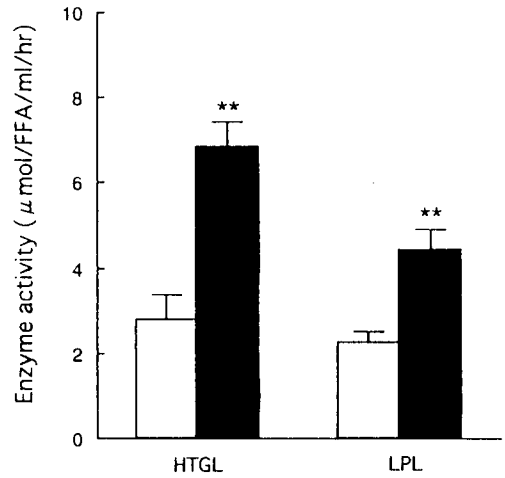

Fig. 3. Effect of OKGK on Hepatic triglyceride lipase and Lipoprotein lipase activities. Postheparin plasma was prepared from normal rats and rats treated with 1.38 g/kg of OKGK for 2 weeks as described in Materials and methods. To normal rats, water were given instead of OKGK. Open column: normal, closed column: OKGK. Values represent mean ± S.E. of 5 rats. ** $p < 0.01$ vs. control group.

Effect of OKGK on lipid synthesis by hepatocytes.

OKGK (1.38 g/kg/day) reduced the incorporation of [2-^{14}C] acetic acid into TG and PL by 37.8 and 29.8%, respectively, while no inhibitory effect was observed in FA synthesis (Table 2). Furthermore, the incorporation of [^{3}H] oleic acid into TG and PL in OKGK-treated group was significantly suppressed by 31.7 and 25.4%, respectively (Table 3). In this experiment, [2-^{14}C] acetic acid was incorporated into TG and PL via glycerol-3-phosphate or fatty acid and [^{3}H] oleic acid was directly incorporated into them following the conversation to [^{3}H] oleoyl-CoA. Actually, the incorporation of [2-^{14}C] acetic acid or [^{3}H] oleic acid into TG and PL in isolated rat primary cultured hepatocytes prepared from OKGK-treated rats was suppressed at the same degree. Furthermore, fatty acid and cholesteryl ester syntheses were not affected by OKGK treatment (data not shown). These results indicated that OKGK treatment seemed likely to modify the common pathway for TG and PL syntheses without the effect on glycerol-3-phosphate and fatty acid syntheses, although the precise mechanism of their suppression was not obvious at present.

Effect of OKGK on HTGL and LPL activities.

OKGK suppressed the increasing of serum TG level in both hypertriglyceridemic and hypercholesterolemic rats, suggesting a possibility that it stimulates TG degradation by LPL or HTGL. In fact, HTGL activity in OKGK-treated rats was found to be significantly higher than that in normal rats and LPL activity was also higher in OKGK-treated rats than in normal rats (Fig. 3). LPL and HTGL are known to contribute the regulation of lipoprotein metabolism. LPL is an enzyme responsible for hydrolysis of the triglyceride-rich core of circulating chylomicrons and VLDL (Nilsson-Ehle et al., 1980) and HTGL mediates the conversion of IDL to LDL (Demant et al., 1988) and of HDL_2 to HDL_3 (Mowri et al., 1992). Disruption of LPL gene in mice elevates serum TG (Coleman et al., 1995). In contrast, transgenic mice that overexpressed HTGL gene did not appear to influence lipoprotein metabolism when fed with a normal chow and further HTGL deficient mice do not develop marked hypertriglyceridemia or accumulation of β-VLDL, although they increase plasma cholesterol and phospholipids attributable to increased HDL levels (Homanics et al., 1995). These observations reveal that LPL is intimately implicated in the metabolism of TG-rich lipoprotein such as VLDL and chylomicrons and has suggested that the antitriglyceridemic action shown by OKGK is likely to depend on the enhanced LPL activity. LPL is secreted by parenchymal cells of various extrahepatic tissues, mainly from muscles, macrophages and adipose tissue and HTGL is exclusively synthesized in the liver (Jansen et al., 1979; Jansen et al., 1978). In short, there are possibilities that OKGK exerts its effect on various tissues to stimulate LPL and HTGL synthesis and that it modulates both enzyme activities although their posttranslational regulation could not be clarified. So far some plant ingredients such as saikosaponin, glycyrrhizin, ginsenoside were reported to show antihyperlipidemic action and among them are flavonoids, plant phenol, ginsenoside and β-sitosterol which were contained in *Astragali* radix, *Cinnamomi* cortex, *Paeoniae* radix and *Ginseng* radix rubra of OKGK. However, it remains to be clarified whether these well known ingredients are able to explain the action of OKGK. Finally, we conclude that OKGK is effective for the treatment of hypertriglyceride-

mia which is induced by endogenous and exogenous TG and that its antihypertriglyceridemic action is in part due to the inhibition of TG synthesis in liver and the enhanced HTGL and LPL activities.

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