Effect of pravastatin on glucose metabolism in HK-2 cells and mice

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Effect of pravastatin on glucose metabolism in HK-2 cells and mice

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This certifies that the Doctoral dissertation of
Yong Pyo Lee is approved.

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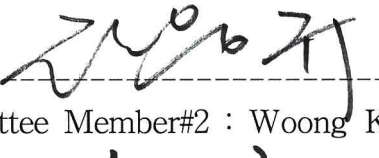

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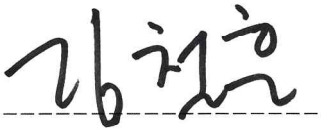

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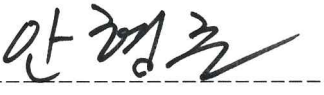

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One of my personal wishes I had long prayed for came true and started to attending church worship every Sunday
30 years after graduation, I thought this may be the perfect opportunity to start my new career and enlisted graduated-course. It took me 8 years to complete my full course and many new ideas and knowledge have opened my eyes and heart.
“Marvelous”
This word occurs in my heart over and over again during 8 years in college.
I would like to express my sincere appreciate to Prof. Yu Seun Kim, who gave excellent scholar’s advice on transplantation while studying and experiencing. Professor Myoung Soo Kim, who always gave the bold support and Professor Beom Seok Kim, who encouraged research interests and fulfilled the thesis. I am also grateful to Yuri Cho and all researchers who provided the best technical support in the laboratory.
Last but the least, I always thank my wife, So-Young Kim, for encouraging me and supporting me. This paper cannot be completed without her support and love.

10 June 2019

<TABLE OF CONTENTS>

LIST OF FIGURES

<ABSTRACT>

Effect of pravastatin on glucose metabolism in HK-2 cells and mice

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Background: Recent reports have revealed that statins are associated with increased incidence of diabetes. Although several mechanisms have been proposed, the role of the kidney glucose metabolism under statin treatment is still unclear.

Methods: HK-2 and HepG2 cells, cultured under high or normal cholesterol conditions, were treated with statins (pravastatin, rosuvastatin, atorvastatin, and fluvastatin). Both gene and protein expression of the enzymes involved in glucose metabolism (glucose 6 phosphatase [G6PC], phosphoenolpyruvate carboxykinase [PEPCK], pyruvate kinase [PKLR], phosphofructokinase [PFK]) were measured. C57BL/6 mice fed either a normal or a high-fat diet were treated with pravastatin and the above-mentioned enzymes were evaluated both the kidney and liver.

Result: A lactate dehydrogenate assay revealed that atorvastatin and fluvastatin exhibited direct cytotoxicity towards HK-2 cells. In HK-2 cells treated with pravastatin under high-cholesterol condition,

the protein expression of only PKLR decreased dose dependently 0.65 ± 0.03 and 0.41 ± 0.07 after treatment with 2 mM and 4 mM pravastatin, respectively). In contrast, the protein expression of PEPCK in HepG2 cells increased significantly, while that of PKLR, PFK-1, and G6PC remained unchanged. Gene expression of PKLR in the pravastatin-treated kidneys from high-fat diet-fed mice decreased to 66 ± 0.02 when compared with that of the control, and the overall protein expression level also markedly decreased. In the kidneys of normal-fat diet-fed mice, gene expression of PFK and PEPCK tended to increase, while that of PKLR and G6PC remained unchanged. In contrast, no changes were found in the liver for all four enzymes. The protein expression level of only PKLR decreased when treated with pravastatin, with no detectable changes in the expression of other proteins. PKLR activity in the kidney decreased to 50.1 ± 6.5 and 38.2 ±10.1 at 2 and 4 weeks of treatment, respectively. No change in enzyme activity was found in the liver. Blood glucose levels at 2 and 4 weeks of treatment increased by about 45 ± 9% and 10 ± 8%, respectively, when compared with those of the control.

Conclusion: We found that pravastatin decreased PKLR activity in the kidney. This change may be associated with the diabetogenic effect of statins. Further studies should be considered to elucidate the precise mechanism by which statins affect PKLR activity in the kidney.

Key words : pravastatin, diabetogenesis, kidney

Effect of pravastatin on glucose metabolism in HK-2 cells and mice

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Ⅰ. INTRODUCTION

Statins, which have been extensively used in clinical trials, dramatically reduce the risk of cardiovascular and cerebrovascular diseases.[1,2] However, many doctors have expressed concern about unanticipated responses or adverse effects associated with the increased clinical use of statins. Frequently reported effects include the onset of muscle-related diseases, diabetes, and diseases of the central nervous system, along with various other adverse effects such as reduction in kidney function, tendon rupture, and organic lung diseases.[3~8]

Of these responses, reports that statins increase the incidence of diabetes have received considerable attention, as well as those describing correlations between diabetes and cardiovascular and cerebrovascular diseases. In particular, there have been a number of

studies focusing on the relationship between statins and T2DM. Recent studies have reported on the functional effects of statins on insulin, i.e., activation of a specific immune response and inhibition of functions of insulin. Nevertheless, this claim needs to be further evaluated.

The diabetogenic effect of statins begins with the inhibition of HMG-CoA reductase via activation,[9] which results in the independent or simultaneous elevation of insulin resistance and reduction of β-cell function.[10,11] The reduction of cholesterol by statins causes a reduction in the expression of GLUT4.[12] In addition, disruption of the cell structure, which harbors insulin receptors, may occur.[13] Moreover, cholesterol reduction results in a decline in the phosphorylation on the insulin receptor substrate (IRS). Additionally, lovastatin is known to reduce the expression of GLUT4.[14] These effects subsided after treatment with mevalonate, which indicated that statins might decrease the effect of insulin.[15] In addition, atorvastatin (a hydrophobic statin) interferes with the translocation of GLUT4, resulting in interference of glucose uptake.[16] This is correlated with a reduction in the expression of small GTP-binding proteins such as RhoA and Rab4, which leads to inhibition of the glucose uptake function of GLUT4.[15] However, this symptom was also reversed after treatment with mevalonate.

In other words, statins decreased the amount of isoprenoid compounds, leading to changes in insulin function.

For decades, it has been known that cholesterol-rich lipoproteins

influence β-cell functions. Increased cellular cholesterol loading impairs β-cell function, proliferation and survival. These effects depend on the function of the LDL-R for chlesterol enry and ATP-binding cassette transporters for cholesterol exit.[17~24]

Treatment with lovastatin reduced insulin secretion induced by glucose in mouse pancreatic cells by 50%[25], which was restored by mevalonate. This effect led to a decrease in the concentration of cytosolic G-proteins.[26]

Simvastatin interferes with β-cell's function in the L-type Ca channel, which decreases the cytosolic Ca^{++} levels, resulting in a reduction in insulin secretion.[27] In another study, simvastatin was found to decrease insulin secretion from β-cells in mouse, thus affecting ATP production, and interfere with GLUT2 expression via reduction of its mRNA level, such that glucose uptake is limited.[27]

CoeQ10 also functions as a signaling protein during mitochondrial ATP production, and statins reduce CoeQ10 levels secondary to inhibition of the mevalonate pathway. Thus, statins interfere with ATP production, small G proein dysfunction and decrease exocytosis of insulin, thus resulting in diabetes.[28]

Animal and cell experiments have revealed that statins are involved in activation of various insulin functions in normal cells or β-cells to induce diabetogenic effects. Thus, we conducted a preceding study to understand the effect of statins on gluconeogenesis or glycolysis in the liver. Statins were found to influence diabetogenic effects, and PEPCK and G6PC enzymes, which are involved in gluconeogenesis, increased and demonstrated their effects.[29]

The human kidney accounts for about 20% of glucose production in gluconeogenesis, and up to 300% in cases of severe starvation or diabetes. This results in 1:1 ratio between the liver and kidney for glucose production, indicating that the kidney is an important organ for glucose synthesis.[30] However, only a few studies have been reported on gluconeogenesis in the kidney and its relationship with statins. Thus, the present study was designed to investigate the role of statins in glucose synthesis and glycolysis in the kidney.

II. MATERIALS and METHODS

1. Cell Culture

Human renal proximal tubular epithelial cell line (HK-2) cells immortalized by transduction with human papilloma virus 16 E6/E7 genes were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 (1:1) (Gibco, USA) culture medium containing 10% fetal bovine serum (Gibco, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco, USA). Hepatocellular carcinoma HepG2 cell lines were cultured in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin, and 100mg/ml streptomycin. Cells were treated with statins (rosuvastatin 5 μM, pravastatin 5μM, atorvastatin 5μM, fluvastatin 1μM) and 0.1 μg/ml 25-hydroxycholesterol.

2. Lactate Dehydrogenase (LDH) Cytotoxicity Assays

After 72 h of treatment, the culture medium was aspirated for LDH assay (MK-401, Takara), performed according to the manufacturer's instructions. Absorbance at 492 nm was measured with a microplate reader (iMARK, BioRad). A 1% triton X-100-treated well was used as a positive control. LDH release was expressed as percentage of the absorbance of the positive control.

3. Animals

C57BL/6 mice were fed a high-fat diet with or without pravastatin (0.01%, w/w) for 16 weeks, and were housed under controlled conditions (21 °C ± 2 °C, 60% ± 10% humidity, 12-h light/12-h dark cycle), with free access to food and water. After 1 week of acclimatization, the mice were divided into 3 groups containing 5 mice each (control group, pravastatin for 2 weeks, and pravastatin for 4 weeks). The food given to each treatment group was supplemented with 0.01% (w/w) pravastatin. Food intake and body weight were evaluated three times a week at the same time of day. The mice were anesthetized with 50 mg/kg Zoletil (Zolazepam; Virbac S.A, France). Blood samples were then collected by cardiac puncture. All animal studies were conducted using a protocol approved by the committee for the care and use of laboratory

animals of Yonsei University College of Medicine.

4. Total RNA Isolation

Following medium aspiration, kidney tissues were lysed by adding TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The lysed samples were incubated for 5 min at room temperature and then mixed with chloroform in a tube by vortexing for 15 s. Tubes were centrifuged at 12,000 *g* for 15 min at 4 °C and the supernatants were transferred into new tubes containing isopropanol, which were incubated at room temperature for 10 min. Sample were then centrifuged at 12,000 *g* for 10 min at 4 °C. The supernatants obtained were discarded and pellets were washed in 70% ethanol and lysed in RNase-free water.

5. RT-PCR and Quantitative Real-Time PCR

Total RNA was reverse-transcribed with an M-MLV Reverse Transcriptase (Invitrogen) protocol, and PCR was performed using Bioneer Accupower PCR PreMix (Bioneer, Korea). Expression of target genes G6PC, PEPCK, PFK1, and PKLR was analyzed by qPCR using SYBR Premix ExTaq (Clontech, RR420A) and gene-specific primers designed from sequences submitted to the NCBI nucleotide sequence database. Transcript values and Ct values were normalized against GAPDH and expressed as fold differences vs. controls.

6. Immunoblotting

Protein extracts from kidney tissue were prepared using radioimmunoprecipitation assay (RIPA) buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholic acid, and 0.1% SDS. Proteins were boiled for 5 min, separated by 10–15% SDS-PAGE, and blotted onto polyvinylidene difluoride membranes. Proteins were detected using the following primary antibodies: hexokinase, pyruvate carboxylase, G6PC, PEPCK (Cell Signaling Technology, Beverly, MA, USA), and β -actin (1:10,000) (Sigma). Protein bands were detected using an Immobilon Western Chemiluminescent HRP substrate kit (Millipore Corporation, Billerica, MA, USA).

7. Pyruvate Kinase (PK) Assay

Pyruvate Kinase (PK) Assay Kit (ab83432, Abcam, UK) was used to determine PK activity following the manufacturer's instructions.

8. Serum Glucose Measurement

Blood samples were collected in microcentrifuge tubes and centrifuged to obtain serum, which was divided into aliquots and stored at -80 °C for subsequent assays. Serum glucose (mmol/L) was determined using a portable glucometer (Medisense Companion

2 meter, Medisense Inc., Waltham, MA) according to the manufacturer's instructions.

9. Statistical Analysis

Results were presented as means ± SEMs. One-way ANOVA and the post hoc Student's t-test were used to determine the significances of differences between means. Statistical significance was accepted for P values <0.05, and the analysis was conducted using GraphPad Prism software.

Ⅲ. RESULTS

1. Cytotoxicity of statins in HK2 cells under high-cholesterol conditions

We first examined the cytotoxic effect of co-treatment with statins and 25-hydroxycholesterol. We treated HK-2 cells with statin only or statin and 25-hydroxysterol. After 72 h of treatment, we measured cell death using the LDH assay. Treatment with either statins or 25-hydroxycholesterol did not induce cytotoxicity. Treatment with both hydrophilic statins (rosuvastatin, pravastatin) and 25-hydroxycholesterol did not induce cell death. However, co-treatment with lipophilic statins (atorvastatin, fluvastatin) and 25-hydroxycholesterol induced substantial HK-2 cell death (Fig. 1).

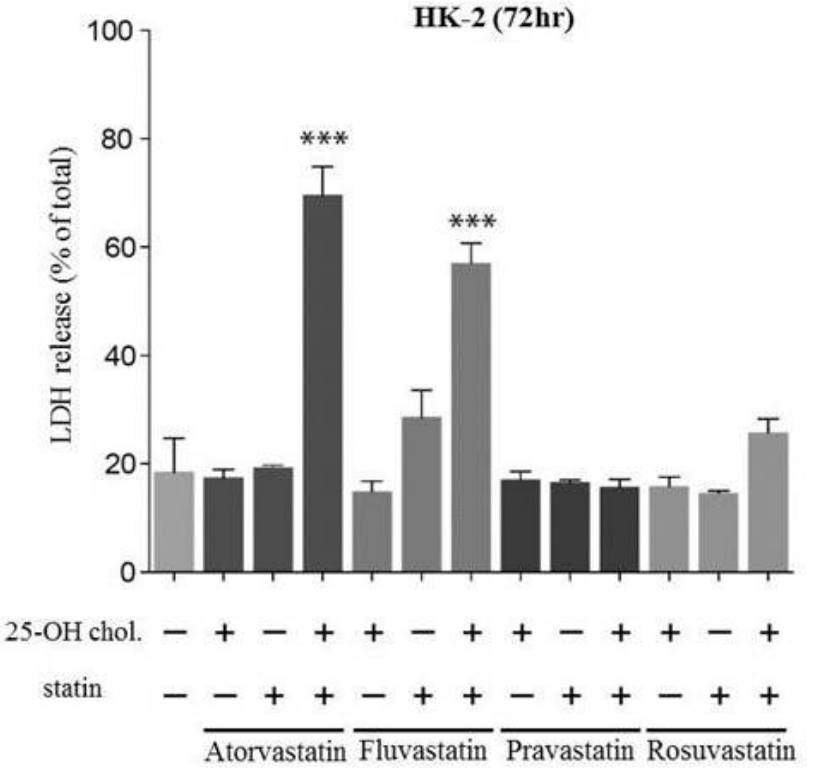


Figure 1. HK-2 cells were co-treated with statin and 25-hydroxycholesterol for 72hr and cell viabilities were measured using a LDH assay. *** P<0.001 versus no treatment group, one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test

2. Effects of pravastatin on glucose metabolism-related enzymes in cells under high-cholesterol conditions

To understand the effect of pravastatin on enzymes involved in glucose metabolism in kidney tubule cells and in the liver under high-cholesterol condition, both HK-2 and HepG2 cells were treated with 1, 2, or 4 μM pravastatin for either 24 or 48 h, and then expression of PKLR, PFK-1, PEPCK, and G6PC proteins was examined by western blotting. HK-2 cells showed no change in the levels of PFK-1, PEPCK, and G6PC proteins depending on pravastatin level and treatment time; however, only PKLR levels decreased in a dose-dependent manner after 48 h treatment (Fig. 2A, B). For PKLR, while HepG2 cells exhibited no change (Fig. 3A, B), HK-2 cells exhibited significant reductions of 0.65 ± 0.03 and 0.41 ± 0.07 after treatment with 2 mM and 4 mM pravastatin at 48 h, respectively, when compared with the control (Fig. 2B). In HepG2 cells, expression of PEPCK increased, while that of PKLR, PFK-1, and G6PC remained unchanged (Fig. 3B).

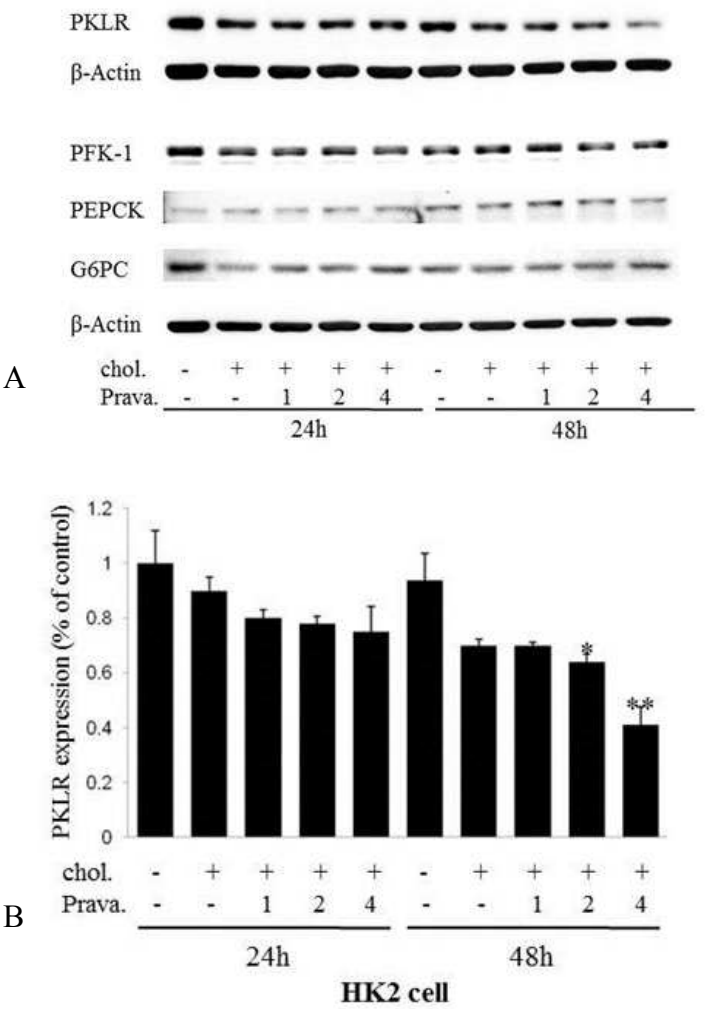


Figure 2. Effects of Pravastatin on glucose metabolism enzyme in high-cholesterol treated HK-2 cells

(A) HK-2 cells were treated with 1, 2, or 4 μM pravastatin plus 25-hydroxycholesterol for 24 hours or 48 hours. Total protein lysates were western blotted. (B) Bar graphs represent quantitative differences in expressions of PKLR. Results are means ± SEMs (n=3). **P*<0.01,***P*<0.001 vs. CTRL

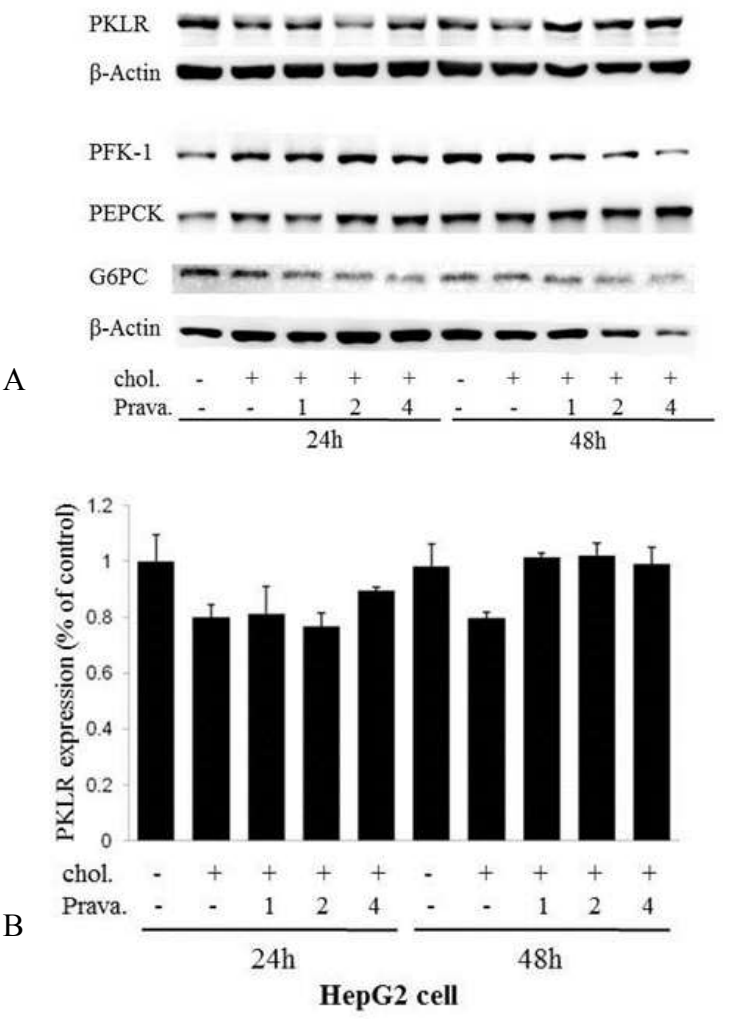


Figure 3. Effects of Pravastatin on glucose metabolism enzyme in high-cholesterol treated HepG2 cells

(A) HepG2 cells were treated with 1, 2, or 4 μM pravastatin plus 25-hydroxycholesterol for 24 hours or 48 hours. Total protein lysates were western blotted. (B) Bar graphs represent quantitative differences in expressions of PKLR. Results are means ± SEMs (n=3). **P*<0.01,***P*<0.001 vs. CTRL

3. Effects of pravastatin on PKLR expression in high-fat diet-fed mice

C57BL/6 mice were fed a high-fat diet with or without a pravastatin (0.01%, w/w) for 16 wk. When gene expression of PKLR in the kidney was tested by real-time PCR, the level of expression greatly decreased to 0.66 ± 0.02% when compared with that of the control (Fig. 4), and its overall protein expression level also markedly decreased, though there were deviations among individuals (Fig. 5A). The level of protein expression decreased to 0.56 ± 0.28% when compared with that of the control (Fig. 5B) These results agree with the results obtained using the HK2 cell line, and show that pravastatin reduce PKLR expression in kidney tubular cells.

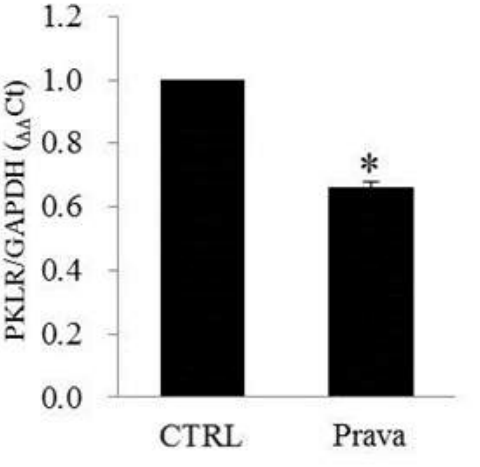
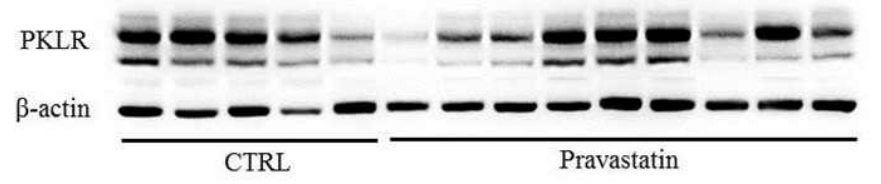
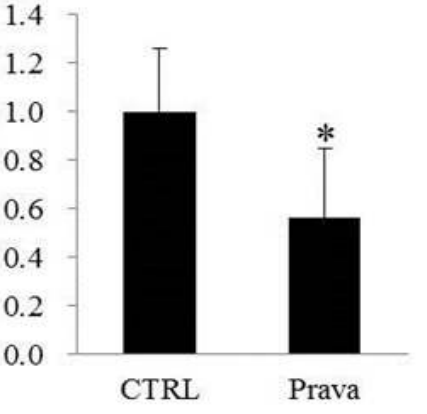


Figure 4. Effects of Pravastatin on PKLR gene expression in high fat diet mice model

To quantify PKLR gene expression in high fat diet mice model, mRNA levels were assessed by qPCR. Results are the means ± SEMs of three independent experiments. **P*<0.01 vs. the untreated control (CTRL). GAPDH was used as an internal control to normalize gene expressions.



A



B

Figure 5. Effects of Pravastatin on PKLR protein expression in high fat diet mice model

(A) Kidney tissue lysates (40 μg) were loaded onto gels and immunoblotted. (B) Bar graphs represent quantitative differences in protein expressions of PKLR. Results are means ± SEMs (n=3). **P*<0.01,vs. CTRL. In high-fat diet mice model, PKLR protein levels were progressively reduced by pravastatin treatment. β-actin was used to confirm equal loading.

4. Effects of pravastatin on glucose metabolism-related enzymes in HK2 and HepG2 cells

From the above results, pravastatin was found decrease PKLR expression in the kidney under high-cholesterol or high-fat conditions. To test the effect of pravastatin alone on the kidney, both HK-2 and HepG2 cells were treated with 1, 2, or 4 μM pravastatin, and then gene and protein expression of PKLR, PFK, PEPCK, and G6PC were investigated. HK-2 cells showed higher gene expression of PKLR, while that of PFK, PEPCK, and G6PC transcripts remained unchanged (Fig. 6A). In HepG2 cells, pravastatin induced no significant changes in gene and protein expressions of PKLR, PFK, PEPCK, and G6PC (Fig. 7A, B). The protein expression levels of PKLR in HK-2 cell were decreased, while those of other enzymes remained unchanged (Fig. 6B). In summary, treatment with pravastatin alone induced more prominent changes in the expression of PKLR in HK-2 cells than those observed in high-cholesterol or high-fat conditions, suggesting that the elevation of PKLR gene expression is attributable to a compensatory effect that results in reduction of protein levels.

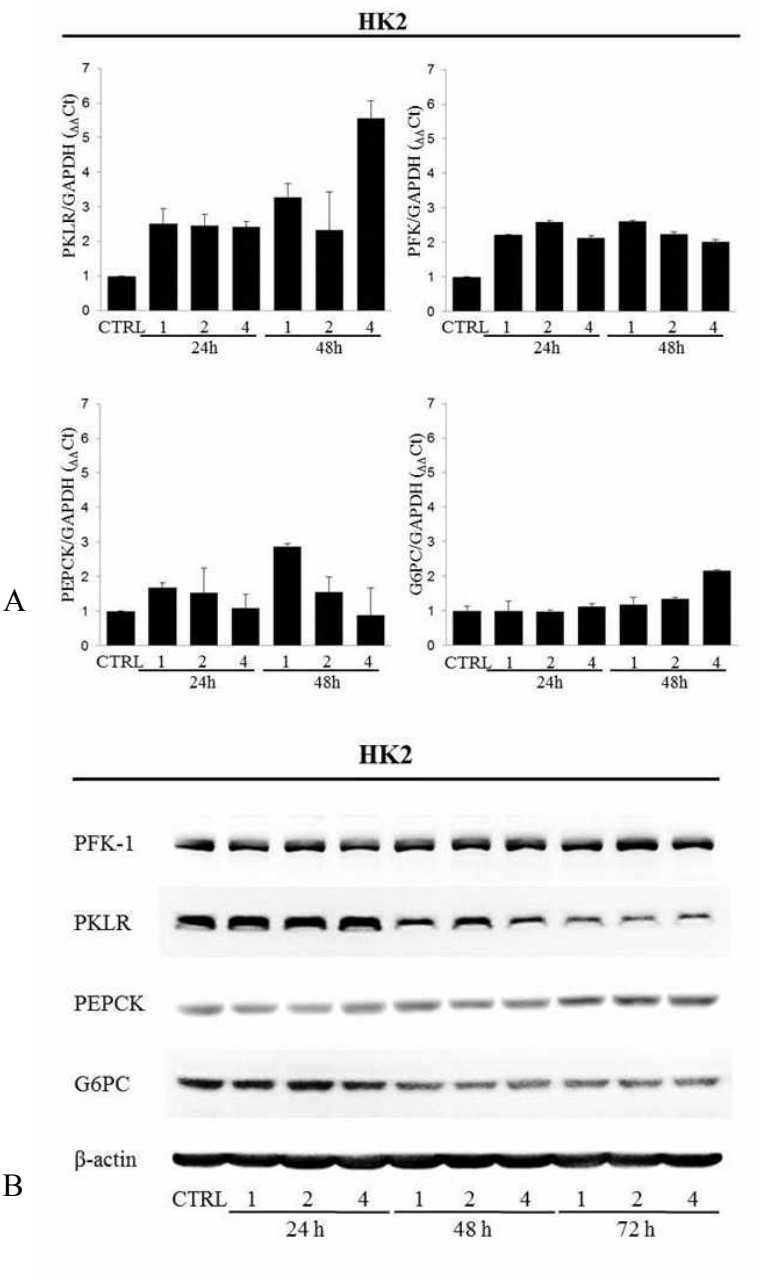


Figure 6. Effects of Pravastatin treatment on glucose metabolism enzyme in HK2 cell

HK-2 cells were treated with 1, 2, 4 μM pravastatin for 24h, 48h, or 72h. (A) The mRNA expressions of PFK1, PKLR, PEPCK and G6PC in HK-2 cells were evaluated by qPCR. GAPDH was used as an internal control to normalize gene expressions. (B) Cell lysates (40 μg) were loaded onto gels and immunoblotted. In HK-2 cells, PKLR protein levels were progressively reduced by pravastatin treatment. β-actin was used to confirm equal loading.

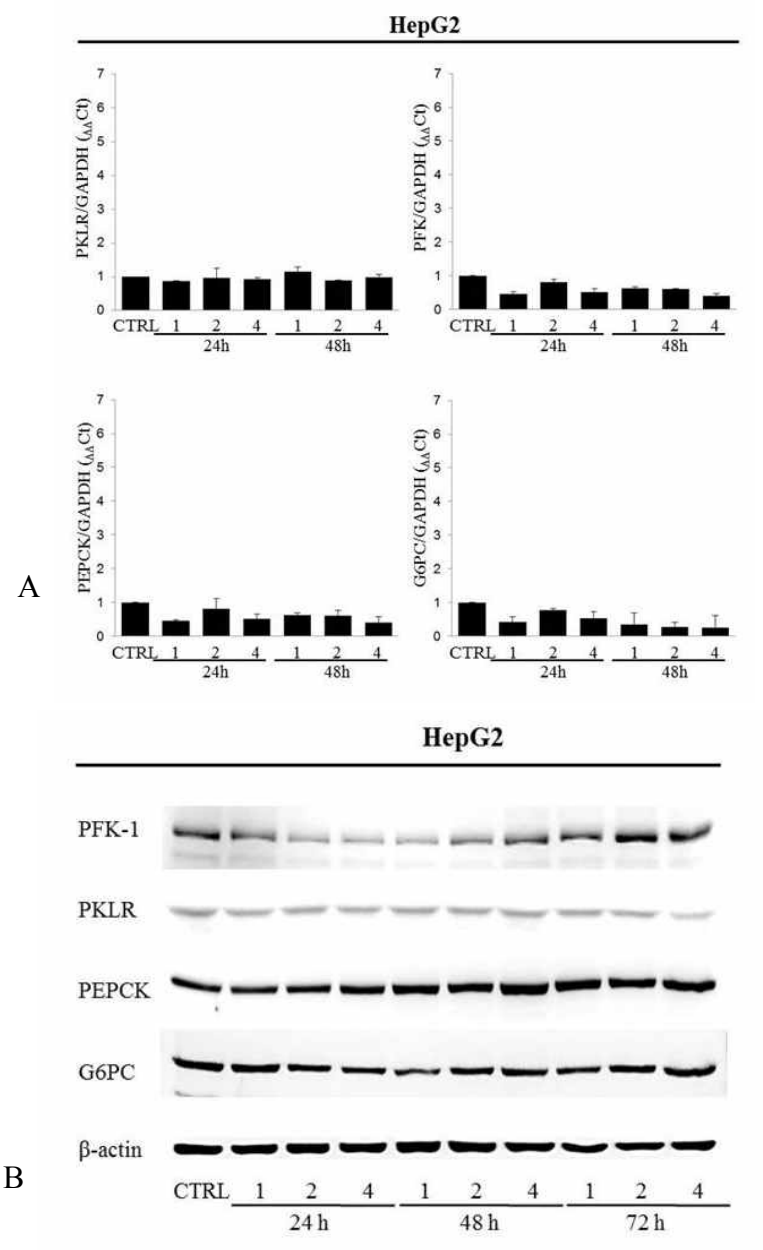


Figure 7. Effects of Pravastatin treatment on glucose metabolism enzyme in HepG2 cell

HepG2 cell were treated with 1, 2, 4 μM pravastatin for 24h, 48h, or 72h. (A) The mRNA expressions of PFK1, PKLR, PEPCK and G6PC in HepG2 cells were evaluated by qPCR. GAPDH was used as an internal control to normalize gene expressions. (B) Cell lysates (40 μg) were loaded onto gels and immunoblotted. In HepG2 cells, PKLR protein levels were not reduced by pravastatin treatment. β-actin was used to confirm equal loading.

5. Effects of pravastatin on glucose metabolism-related enzymes in C57BL/6 mice

To investigate the effect of pravastatin on enzymes involved in glucose metabolism in the kidney and liver, 8-week-old C57BL/6 mice were reared for 2 or 4 weeks with a formula feed containing 0.01% pravastatin. There was no difference in changes of weight between the experimental groups during the periods. Gene expression of PFK and PEPCK tended to increase in the kidney, whereas that of PKLR and G6PC remained unchanged (Fig. 8A). No changes were observed in the expression of all four enzymes in the liver (Fig. 9A, B). The protein expression levels of only PKLR, obtained from the data from the cell lines, decreased, whereas no detectable changes were observed in other proteins (Fig. 8B).

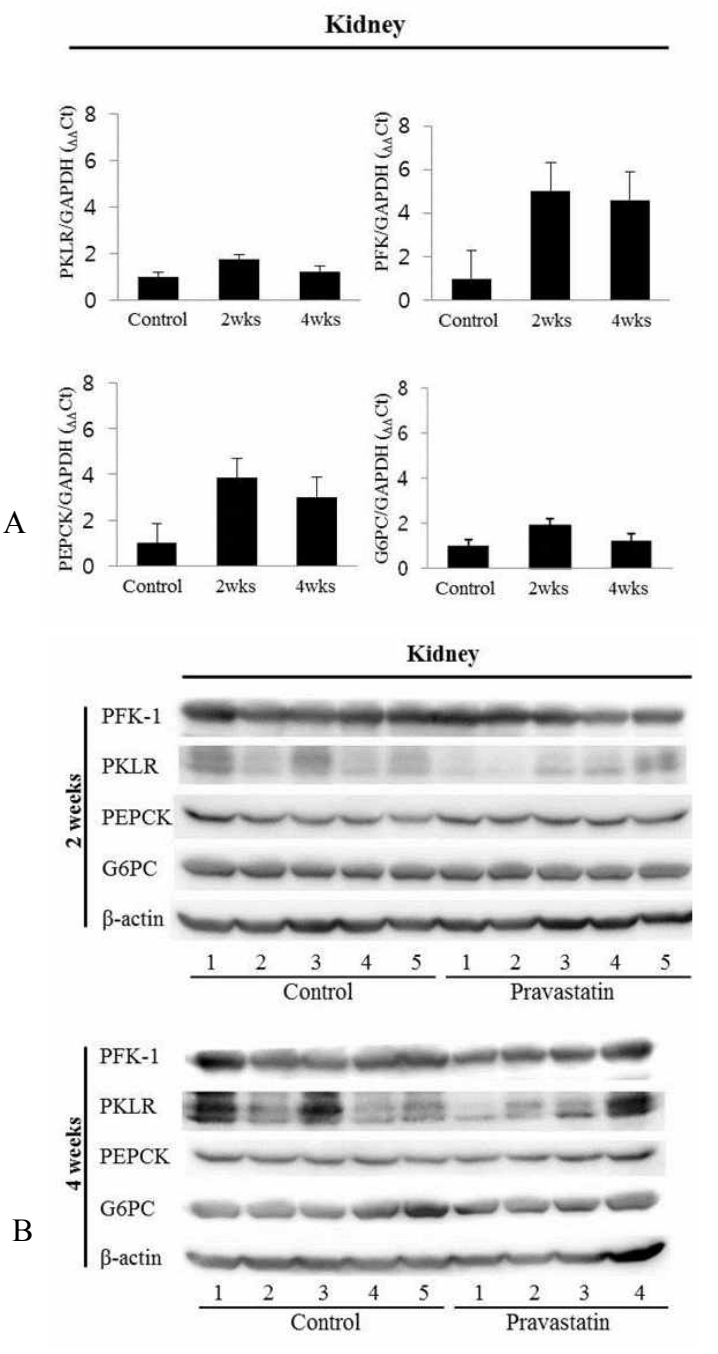


Figure 8. Effects of Pravastatin treatment on glucose metabolism

enzyme of kidney in C57BL/6 mice

C57BL/6 mice were fed a pravastatin (0.01%, w/w) for 2wk or 4 wk (n=5). (A) The mRNA expressions of PFK1, PKLR, PEPCK and G6PC in kidney were evaluated by qPCR. GAPDH was used as an internal control to normalize gene expressions. (B) Tissue lysates (40 μg) were loaded onto gels and immunoblotted. β-actin was used to confirm equal loading.

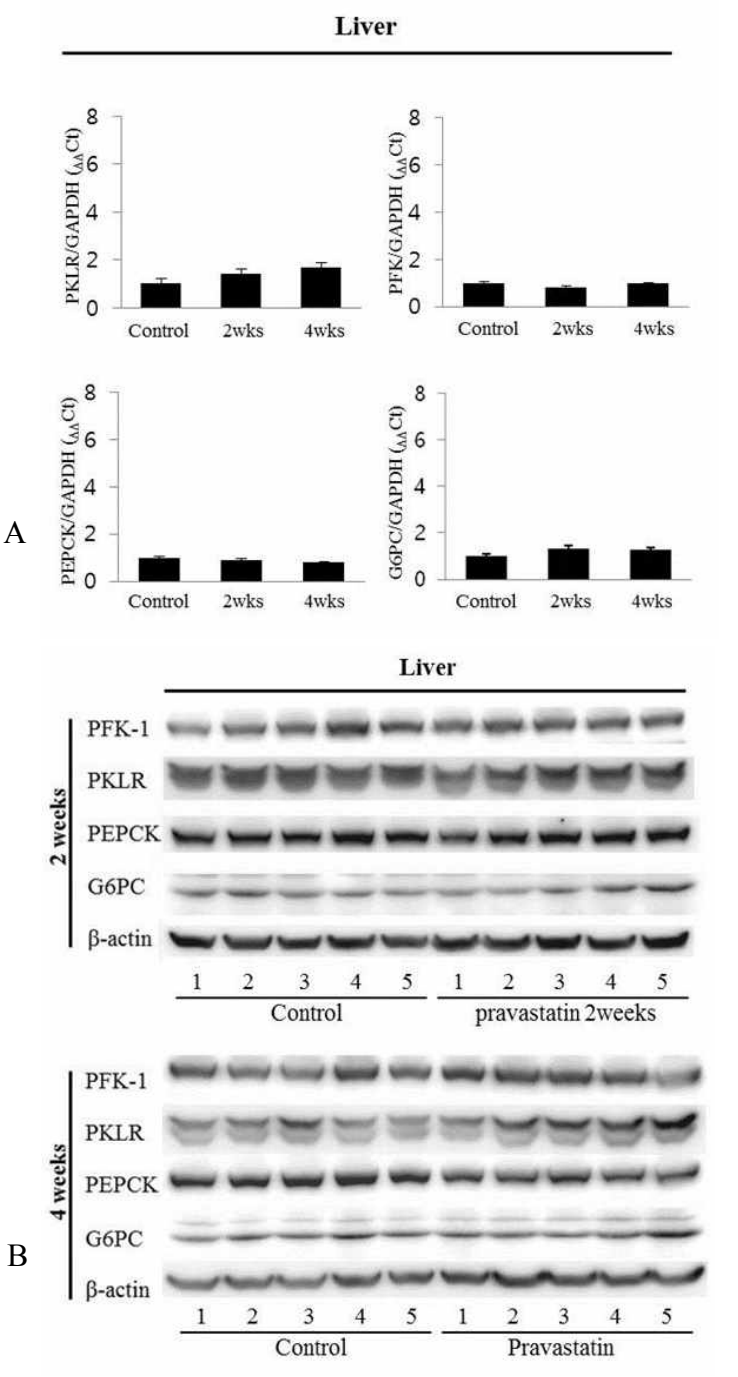


Figure 9. Effects of Pravastatin treatment on glucose metabolism

enzyme of liver in C57BL/6 mice

C57BL/6 mice were fed a pravastatin (0.01%, w/w) for 2wk or 4 wk (n=5). (A) The mRNA expressions of PFK1, PKLR, PEPCK and G6PC in liver were evaluated by qPCR. GAPDH was used as an internal control to normalize gene expressions. (B) Tissue lysates (40 μg) were loaded onto gels and immunoblotted. β-actin was used to confirm equal loading.

6. Alteration of PKLR activity by pravastatin in C57BL/6 mice

Pravastatin was found to reduce the expression of PKLR. To understand the actual effect of pravastatin on PKLR activity, a PKLR activity assay was performed. The group treated with pravastatin for 2 weeks showed a reduction in PKLR activity in the kidney from 50.1 ± 6.5% to 38.2 ±10.1% after 4 weeks of treatment (Fig. 10A). In contrast, no change was observed in the liver (Fig. 10B). In addition, glucose levels in blood collected at the time of sacrifice were measured to identify the effect of PKLR reduction on blood glucose levels in the kidney. Glucose levels of the groups treated with pravastatin for 2 and 4 weeks increased by about 45 ± 9% and 10 ± 8%, respectively, when compared with those of the control (Fig. 11).

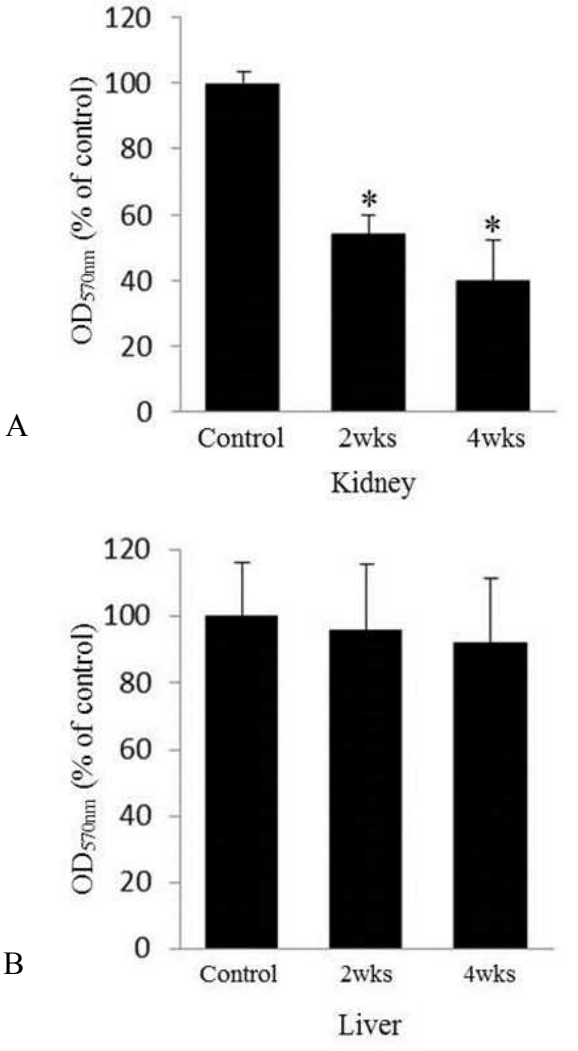


Figure 10. Alteration of PKLR activity by pravastatin treatment on C57BL/6 mice

In the PKLR activity assay, PEP and ADP were catalyzed by PKLR to generate pyruvate and ATP. The generated pyruvate is oxidized by pyruvate oxidase to produce color at 570nm. (A) Kidney (B) Liver

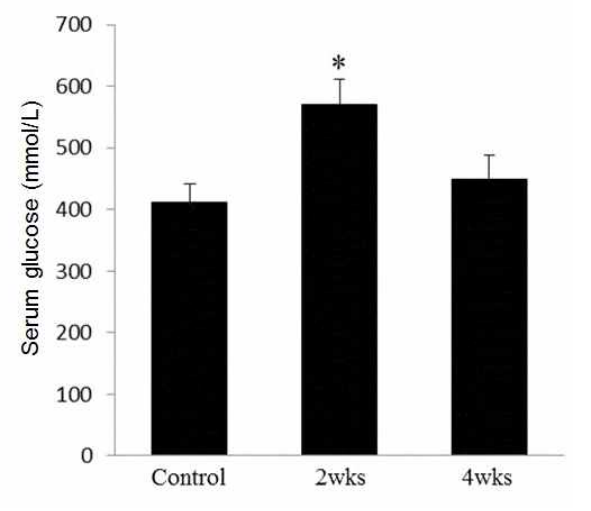


Figure 11. Effects of Pravastatin on serum glucose in C57BL6 mice

Blood glucose level in mice were measured after 2wk and 4wk. Serum glucose (mmol/L) was determined using a portable glucometer.

Ⅳ. DISCUSSION

The present study investigated the effect of a statin on PFK, PKLR, PEPCK, and G6PC genes in the kidney, which are involved in glucose metabolism. When cells were treated with pravastatin after cholesterol pretreatment, only PKLR showed significant reduction in both gene and protein expression levels in HK-2 cells. Pravastatin was found to interfere with PKLR production, which resulted in a diabetogenic effect. To understand the individual function of pravastatin in the kidney, cells were tested without cholesterol treatment. HK-2 cells showed a significantly higher expression of the PKLR gene, whereas its protein expression was markedly lower, thus hinting that the reduced protein level increased compensatory gene expression. In contrast, HepG2 cells showed no detectable change, which suggested that statins decrease PKLR levels specifically in the kidney. This reduction of PKLR by pravastatin was more prominent in the in vivo study.

Gluconeogenesis in the kidney occurs in the proximal tubule using lactate, glycerol, and amino acids.[31] Glucose uptake are of different types, depending on S_1, S_2, and S_3 parts of the proximal tubule. In detail, S1(S2) parts, with high flux low affinity, uptake most of produced glucoses, whereas S3 part with low flux high affinity, uptakes the remaining 10% of glucose.[32] In cases of unique glucose metabolism and transport in the kidney, and in diabetes

conditions in which glucose production increases along with changes in the uptake process, use of statins should have a significant effect in patients who have already had diabetes or had prodrome of diabetes.

A preceding study on statins and diabetes in the liver, which accounts for most of glucose metabolism, investigated the relationship between statins and molecules involved in gluconeogenesis and glycolysis, such as GCK, PKLR, PEPCK, and G6PC enzymes. The expression of PEPCK and G6PC, involved in gluconeogenesis, increased due to the effect of statins, which tended to induce gluconeogenesis in the liver. Thus, it was concluded that statins could contribute to the induction of diabetes.[29]

In summary, statins, unlike in the liver, were found to act on PKLR in the kidney. PKLR is an enzyme involved in glycolysis, and not in gluconeogenesis. The reduction of PKLR interfered with glycolysis, resulting in higher glucose levels. In other words, statins increased gluconeogenesis in the liver and reduced glycolysis in kidney, resulting in the same effect. Hence, the effects of statins on enzymes involved in glucose metabolism vary depending on the organ; therefore, it is important to further investigate different functions of statins in the body.

Ⅴ. CONCLUSION

In conclusion, statins were found to be involved in glucose metabolism in various ways, affecting T2DM expression or aggravating clinical symptoms. In particular, an HMG CoA reductase inhibitor functioned to decrease the production of mevalonate, leading to a continuous reduction of cholesterol synthesis, and a subsequent reduction of isoprotenoid products (Dolichol, FPP, GGPP, CoQ10), whereby statins were involved in the control of secretion and functions of insulin, as shown in animal experiments. However, a full elucidation of these procedures could not be reflected in the present study, and could be considered as a limitation.

In the future, the adversed effect of statins should be further studied. And it is recommended that statins should be continuously used rather than limited because of minor adverse drug effects, which could be treated depending on individual cases.

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ABSTRACT(IN KOREAN)

HK-2 세포와 생쥐에서 pravastatin이 신장 당대사에 미치는 영향

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이 용 표

배경: 지질대사개선제인 statin 계열의 약들이 당뇨의 유병율을 높인다는 보고들이 늘고 있으며 그 기전에 대한 다양한 설명들이 나오고 있으나, statin계열의 약물 투여 시 나타나는 당대사의 변화에 대한 콩팥 내에서의 역할은 불분명한 실정이다.

방법: 고농도 콜레스테롤 환경에서 배양된 HK-2 세포와 HepG2세포를 statin계 약물들 (pravastatin, rosuvastatin, atorvastatin, fluvastatin)로 처치 후 당 대사과정에 관여하는 4개의 효소 (G6PC, PEPCK, PKLR, PFK)의 유전자 및 단백질 발현의 변화를 관찰하고 대조군과 비교하였다. 고지방식이요법을 시행한 C57BL/6 쥐를 pravastatin처치 후 실험 쥐의 간과 콩팥에서 위에서 언급한 4개의 효소의 유전자 및 단백질 발현의 변화를 관찰하고 대조군과 비교하였다.

결과: Lactic dehydrogenase assay결과 atorvastatin과 fluvastatin은 직접적인 세포독성을 HK-2 cell에 주는 것으로 나

타났다. 고콜레스테롤 환경에서 pravastatin을 처치한 HK-2 cell에서 PKLR만이 pravastatin 용량에 비례하여 단백질발현이 감소되는 것으로 나타났다 (2mM의 pravastatin 처치 시, 0.65 ± 0.03; 4mM의 pravastatin 처치 시, 0.41 ± 0.07). 이와 다르게 같은 조건의 HepG2 cell의 경우 PEPCK의 단백질 발현은 높아졌으며 PKLR, PFK-1, G6PC의 변화는 없었다. 고지방 식이요법의 쥐에 pravastatin처치 후 콩팥에서 PKLR유전자 발현의 변화가 대조군에 비해 의미 있게 감소함을 보여주었다 (66 ± 0.2). 단백질 발현의 경우도 pravastatin을 처치한 경우 대조군에 비해 PKLR이 의미 있게 감소함을 보여주었다. 정상 콜레스테롤 상태에서 배양한 HK-2 cell과 HepG2 cell에 pravastatin 처치 후 4개의 효소의 유전자 및 단백질 발현을 각각 관찰하여 대조군과 비교하였다. HK-2 cell에서 PKLR의 유전자 발현이 증가되었고 다른 효소들의 변화는 관찰 할 수 없었다. HepG2 cell에서 4가지 효소의 변화는 없었다. 단백질 발현의 경우 HK-2 cell에서 PKLR의 감소가 pravastatin의 노출시간에 비례하여 나타났으며 다른 효소들의 변화는 관찰 할 수 없었다. HepG2 cell에서 4가지 효소의 변화는 없었다.

정상식이요법을 시행한 쥐에 pravastatin처치 후 콩팥과 간에서의 4가지 효소의 유전자 및 단백질 발현을 관찰 하고 대조군과 비교하였다. 유전자 발현의 경우 콩팥에서 PFK와 PEPCK의 발현이 증가되었고 간에서의 변화는 없었다. 그러나 단백질 발현의 경우 콩팥에서는 PKLR만이 처치기간에 관계없이 감소하였고 다른 효소의 변화는 없었으며 간에서의 효소 변화는 관찰 할 수 없었다. 이러한 PKLR유전자 및 단백질 발현이 실제적으로 쥐의 생체 내에서 어떤 식으로 나타난 가를 알기 위해 PKLR의

activity assay를 시행하였고 그 결과 콩팥에서는 PKLR의 활성도가 기간이 길어질수록 대조군에 비해 감소함을 보였고 간에서의 변화는 없었다.

결론: 본 연구를 통하여 pravastatin이 쥐의 콩팥 내에서 PKLR의 활성도를 감소시키는 것을 관찰 하였고, 이 변화가 statin의 당뇨발생 효과를 설명할 수 있는 근거를 마련하였다.

핵심되는 말 : 프라바스타틴, 당뇨유발, 신장