S-9-PAHSA regulates glycolipid metabolism by enhancing autophagy and upregulating PI3K/AKT pathway

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Research Article

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Abstract

Background: The disorders of glucolipid metabolism are prevalent in patients with type-2 diabetes mellitus (T2DM). Palmitic-Acid-Hydroxy-Stearic Acid (PAHSA) had been reported to benefit glucolipid metabolism in diabetic mice. Given the importance of autophagy in T2DM, the aim of the present study was to investigate whether S-9-PAHSA has specific therapeutic effects on diabetics, particularly with regards to autophagy.

Methods: 19-week-old male C57BL6 mice were fed with high-fat-diet for 4 months, and administered with 22.5 mg/kg/d or 5.5 mg/kg/d fatty acids or an equivalent volume of vehicle for 35 days. Glucose levels and weight were determined. Insulin, glycosylated serum protein (GSP), glucagon-like peptide-1(GLP-1) and oxidized modified low-density lipoprotein (ox-LDL) were assessed by ELISA kit. Autophagy-related proteins P62 and Beclin-1in cerebral cortex were detected by western blot. SH-SY 5Y cells were incubated under diabetic environment (glucose 100mmol/L, fatty acid 200μmol/L), and then treated with S-9-PAHSA, R-9-PAHSA and 9-PAHSA. Cell viability, cell proliferation, lactate dehydrogenase (LDH) release and reactive oxygen species (ROS) production were detected by respective kits. Apoptosis was assessed by the TUNEL assay. The protein levels of apoptosis (Cleaved caspase-3, BAX/Bcl-2), autophagy (Beclin1, p62 and LC3II/I) and PI3K/AKT pathway were determined by western blot.

Results: Although S-9-PAHSA did not alter glucose levels, weight, GLP-1 and GSP in mice, high-does S-9-PAHSA improved insulin resistance and lowered ox-LDL. Compared with control group, the up-regulated expression of P62 and down-regulated expression of Beclin1 in diabetic mice were found, and the S-9-PAHSA administration reduced P62. In vitro, S-9-PAHSA increased cell viability and proliferation while reduced LDH release and ROS production, which were better than R-9-PAHSA and 9-PAHSA. S-9-PAHSA could lower the levels of Cleaved caspase-3 and percentage of TUNEL-positive cells. S-9-PAHSA enhanced the expression of LC3II/I under high glucose and fatty acid stimulation. PI3K and p-AKT/AKT ratio under diabetic environment had a downward trend, and S-9-PAHSA might enhance their expression, but there was no statistical difference.

Conclusions: S-9-PAHSA could regulate glycolipid metabolism in HFD-induced T2DM mice through autophagy-associated protein, and exerted protective effects on diabetes by the regulation of autophagy, reducing oxidative stress, partially activating PI3K/AKT signaling pathway.

Introduction

Type-2 diabetes mellitus (T2DM) is characterized by increased glucose levels in the blood, insulin resistance, and metabolic abnormalities[1, 2]. The imbalance of glycolipids homeostasis could easily lead to inflammatory, apoptosis, oxidative stress, and autophagy, resulting in cell dysfunction.

In 2014 Yore et al.[3] reported a new endogenous fatty acid, Palmitic-Acid-Hydroxy-Stearic Acid (PAHSA), which aroused extensive attention because of its anti-diabetic and anti-inflammatory effects. Supplementation of omega-3 fatty acids has beneficial effects on chronic metabolic diseases[4, 5].

PAHSA has similar effects with omega-3 fatty acids, but it is endogenously synthesized in mammals[3]. Decreased levels of endogenous PAHSAs in serum had been found in diabetic patients[6]. Beneficial effects of PAHSAs have been linked to metabolic disorders such as diabetes[3, 7], colitis[8], and nonalcoholic fatty liver disease[9]. Nevertheless, there was also studies reporting no detection of antidiabetic effects after PAHSA treatment in a mice model of diet-induced obesity[10]. We suspected that the configuration of PAHSAs may lead to these contrary studies. There is a great number of isomers of this compound, the highest of which was 9-PAHSA. It's reported that S-9-PAHSA, but not R-9-PAHSA, potentiated glucose-stimulated insulin secretion (GSIS) and glucose uptake[11]. Since different optical activity of substance had differential roles in bioactive lipid function, in this study, we separated 9-PAHSA as S-form stereoisomers and R- form stereoisomers by using chiral method[12].

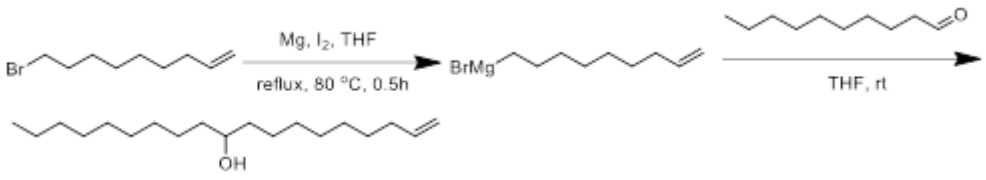
Autophagy plays an integral part in conserving cellular homeostasis by removing cytotoxic proteins and damaged organelles under adverse stress conditions[13, 14]. It had been intensely understood that autophagy functions in T2DM[15], and the autophagic phenotype is associated with oxidative stress[16], apoptosis[17], and inflammation[18]. The mechanism of autophagy is complicated involving critical signaling pathways, such as PI3K III / Beclin-1, AMPK, P53, MAPK / Erk1 / 2, etc[19–22]. The PI3K/AKT pathway plays a significant role in the of autophagic progression. Insulin is a crucial factor controlling blood glucose levels in peripheral tissues also by activating the PI3K/AKT pathway[23]. Notably, PAHSAs may also play a major role in mediating autophagy. The aim of this study was to investigate whether that S-9-PAHSA could improve glycolipid metabolism by enhancing autophagy through upregulation of PI3K/AKT pathway.

Methods

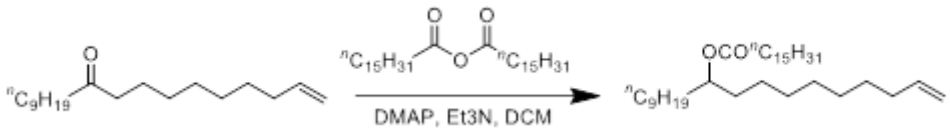
Synthesis of 9-PAHSA and chiral resolution of 9-PAHSA

9-PAHSA and its chiral resolution (S-9-PAHSA and R-9-PAHSA) were synthesized in Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, in accordance with the method described previously by Yore, et al[3]. The procedure for the synthesis was shown in the following:

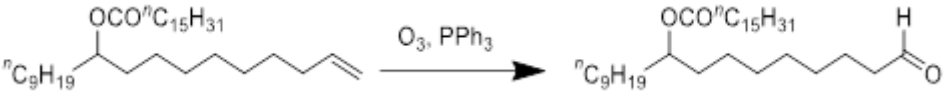
1. Synthesis of nonadec-1-en-10-ol:



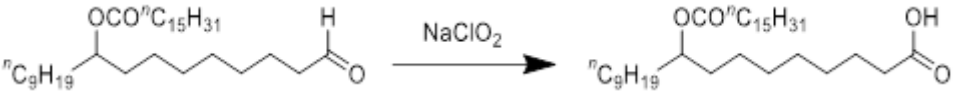
2. Synthesis of nonadec-1-en-10-yl palmitate:



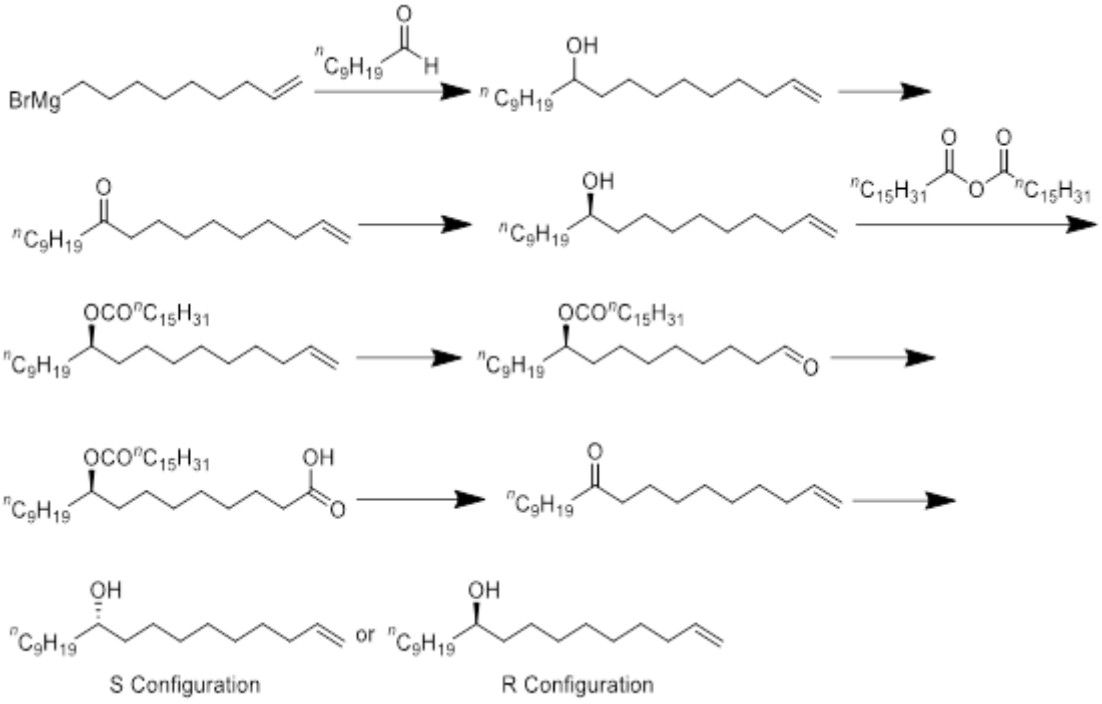
3. Synthesis of 1-oxooctadecan-9-yl palmitate:



4. Synthesis of 9-PAHSA:



5. Synthesis process of chiral 9-PAHSA



Once synthesized, S-9-PAHSA was dissolved in sodium carboxymethyl cellulose (China National Pharmaceutical Group Corporation) for *in vitro* experiments and dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Darmstadt, Germany) for *in vivo* experiments.

Animal grouping and intervention

We purchased 4-week-old wild-type male C57BL/6 mice (25 ± 10g) from Shanghai SLAC Laboratory Animal Co., Ltd. Mice were fed a normal diet until 19 weeks of age. All procedures were carried out according to the National Institutes of Health guide for the care and use of Laboratory animals and approved by animals ethical committee of Fudan University.

24 mice were fed on high-fat diet (HFD) for 4 months and then injected intraperitoneally with streptozotocin (STZ) (35mg/kg/d) for 5 consecutive days. One week later, 18 mice with fasting blood glucose (FBG) higher than 11.0mmol/L were defined as the successful type 2 diabetes mice. The control male C57BL/6 mice with the same age were fed with ordinary diet.

Mice were divided into 4 groups (n = 6 per group): control (Con), vehicle (Veh), low-dose (5.5mg/kg) S-9-PAHSA (LdS), and high-dose (22.5mg/kg) S-9-PAHSA (HdS), and administered with these doses daily by intraperitoneal injection for 5 weeks, and then animals were sacrificed. The experimental process was showed in the Fig. 1.

Enzyme-linked immunosorbent assay (ELISA)

The serum was harvested and insulin, glycosylated serum protein (GSP), glucagon-like peptide-1(GLP-1) and oxidized modified low-density lipoprotein (ox-LDL) were assessed by ELISA kit (Sigma-Aldrich, Darmstadt, Germany). Insulin resistance was assessed using the homeostatic model assessment for insulin resistance (HOMA-IR) from fasting insulin and glucose levels[24]. Glucose levels were determined using Accu-Check active bands (Roche Diagnostics).

Cell culture and detection

We established an *in vitro* diabetic environment in SH-SY 5Y cells under high glucose and fatty acids exposure. SH-SY 5Y cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% FBS, 5% horse serum, 100 μg/ml streptomycin, 100 U/ml penicillin, and incubated at 37°C with 5% CO_2 humidified atmosphere. Cells were separately cultured 5 kinds of medias, including DMEM (control), DMEM with extra high glucose and fatty acid (GF, glucose 100mmol/L, fatty acid 200μmol/L), GF + S-9-PAHSA (30μmol/L), GF + R-9-PAHSA (30μmol/L), GF + 9-PAHSA (30μmol/L). Cell viability, lactate dehydrogenase (LDH) release and reactive oxygen species (ROS) production were detected by CCK-8 assay kit (Beyotime Biotechnology), LDH Cytotoxicity assay kit (R&D systems) and ROS Assay Kit (Beyotime Biotechnology), respectively.

5-bromo-2'-deoxyuridine (BrdU) detection

BrdU proliferation was determined using a BrdU Cell Proliferation Assay Kit (Roche Diagnostics, USA). Cells were seeded in 24-well plates and treated with various medias for 24 h. BrdU solution (100μmol/L) was then added into wells and incubated at 37°C for 2–4 h, after which cell proliferation was recorded. After cell fixation and DNA denaturation, cells were labeled with 200μl of anti-BrdU solution overnight at 4°C, then washed with phosphate buffered solution (PBS). Finally, incubated slides with the secondary antibody (Alexa 594 goat-anti-mouse antibody) and DAPI. $BrdU^+$ cells were observed and photographed under fluorescence microscope. Red fluorescence was positive cell and blue fluorescence was nucleus. $BrdU^+$ cells and total cells were measured with Image J Software.

TUNEL assay

The TUNEL staining was carried out using the Dead End Fluorometric TUNEL System kit (Abcam, Cambridge, UK). DNA which has been labeled with fluorescein could then be analyzed by fluorescence microscopy. TUNEL-positive cells and total cells were measured with Image J Software.

Western blot

Protein quantitative analysis was using western blot, and protein extraction, quantification, and immunoblotting were performed as described previously[25]. In our study, the immobilized proteins were incubated with primary antibodies such as P62 (1:1000, Cell-Signal Technology), LC3(1:1000, Cell-Signal Technology), Beclin1 (1:1000, Cell-Signal Technology), Bcl-2(1:500, Cell-Signal Technology), p-AKT (1:1000, Cell-Signal Technology), AKT (1:1000, Cell-Signal Technology), PI3K (1:1000, Cell-Signal Technology) and β-actin (1:2000, Cell-Signal Technology). See supplementary material for more details.

Statistical Analysis:

Statistical analysis was performed using Prism 7.0 (GraphPad Software, San Diego, USA). All data were expressed as mean ± SEM. Differences between groups were assessed using unpaired t-tests and/or ANOVA with Fisher's LSD multiple comparisons as specified in figure legends. The test level (α) was 0.05.

Results

Chronic treatment of S-9-PAHSA improved insulin resistance and lipid metabolism in diabetic mice.

We evaluated whether chronic treatment of S-9-PAHSA improves the glycolipid metabolisms in T2DM mice. After 35 days of administration, the levels of FBG and GSP were significantly elevated in T2DM mice (Fig. 2a and c, $p < 0.001$, $p < 0.05$), and slightly lower after S-9-PAHSA treatment, although this difference was not statistically significant. Levels of plasma insulin remained unchanged in both T2DM mice and C57BL/6 mice (Fig. 2d, $P > 0.05$). Decreased HOMA-IR was observed in high-dose-S-9-PAHSA group (Fig. 2e, $P < 0.01$), suggesting that the treatment of S-9-PAHSA could improve insulin resistance in HFD-induced T2DM mice. There was no obvious improvement in T2DM mice with regards to GLP-1 secretion (Fig. 2f), however, the serum level of ox-LDL in the T2DM mice was significantly lower after 35 days of high dose S-9-PAHSA treatment (Fig. 2g, $p < 0.05$), suggesting that S-9-PAHSA might exert effects on lipid metabolism and the suppression of lipid peroxidation in T2DM mice to some extent.

Chronic treatment of S-9-PAHSA improved autophagy of cerebral cortex in diabetic mice.

In order to investigate whether the central protective roles of S-9-PAHSA in diabetic individuals is though regulating autophagy, P62 and Beclin-1in cerebral cortex, as classical autophagy-related proteins, were detected by using western blotting analysis. We observed that up-regulating of P62 and down-regulating expression of Beclin-1 in the cerebral cortex of HFD-induced diabetes mouse (Fig. 3, $P < 0.01$, $P < 0.05$ vs control). After the intervention of S-9-PAHSA, P62 was significantly decreased (Fig. 3a, $P < 0.05$), while Beclin1 was slightly increased (Fig. 3b, $P > 0.05$).

S-9-PAHSA alleviated GF-induced injury in SH-SY 5Y cells

We detected the cell viability, LDH release and oxidative stress in SH-SY 5Y cells. The cell viability of GF group was lower than that of control group (Fig. 4a, $P < 0.01$), and S-9-PAHSA treatment significantly increased the SH-SY 5Y cell viability (Fig. 4a, $P < 0.05$); however, R-9-PAHSA and 9-PAHSA treatment had no effect on cell viability (Fig. 4a, $P > 0.05$). The LDH release was also significantly increased in GF group as compared with the control group (Fig. 4b, $P < 0.01$). In addition, the treatment of S-9-PAHSA, R-9-

PAHSA and 9-PAHSA significantly resulted in lower LDH release under diabetic condition compared with GF group (Fig. 4b, $P < 0.0001$, $P < 0.01$, $P < 0.01$). Obviously, S-9-PAHSA treatment lower LDH release compared with R-9-PAHSA (Fig. 4b, $P < 0.05$). As showed in Fig. 4c, we found that the ROS level under diabetic environments was higher than that in control group ($P < 0.001$), and declined after S-9-PAHSA treatment ($P < 0.05$). It's indicated that S-9-PAHSA treatment could alleviate the cells' injury under diabetic condition by decreasing cytotoxicity, and these effects better than R-9-PAHSA or 9-PAHSA.

S-9-PAHSA promoted cell proliferation.

Compared with the control group, $BrdU^+$ cells was markedly lower under diabetic condition (Fig. 4i, $P < 0.001$) and increased after the treatment of S-9-PAHSA, R-9-PAHSA and 9-PAHSA (Fig. 4i, $P < 0.05$). However, there was no statistical difference among S-9-PAHSA, R-9-PAHSA and 9-PAHSA (Fig. 4i, $P > 0.05$).

S-9-PAHSA might reduce the levels of apoptosis-related proteins.

To determine whether S-9-PAHSA alter apoptosis, we detected the levels of apoptosis-related proteins, in SH-SY 5Y cells. The levels of cleaved caspase-3 were significantly decreased after S-9-PAHSA administration, compared with that in GF group (Fig. 5a, $P < 0.01$). Compared with control group, all the levels of BAX obviously decreased in GF group, GF + S-9-PAHSA group and GF + R-9-PAHSA group (Fig. 5b, $P < 0.05$, $P < 0.01$, $P < 0.05$). Under the high glucose and fatty acid environment, the levels of Bcl-2 decreased (Fig. 5c, $P < 0.05$), and increased after S-9-PAHSA intervention (Fig. 5c, $P < 0.01$). Compared with control group, the ratio of BAX/Bcl-2 decreased in GF + S-9-PAHSA group and GF + R-9-PAHSA group (Fig. 5d, $P < 0.01$, $P < 0.05$). However, the ratio of BAX/Bcl-2did not change after S-9-PAHSA treatment (Fig. 5d, $P > 0.05$). Compared with the GF group, percentage of TUNEL-positive cells in the GF + S-9-PAHSA group was invariably lower (Fig. 5h, $P < 0.05$).

S-9-PAHSA might regulate the levels of autophagy-related proteins.

The expression of autophagy-related protein LC3II/I decreased under diabetic condition which was reversed after S-9-PAHSA intervention (Fig. 6a, $P < 0.01$ vs control). Compared with the control group, the expression of Beclin1 in GF group decreased (Fig. 6b, $P < 0.05$), and the Beclin1 increased after S-9-PAHSA intervention, but there was no statistically significant difference (Fig. 6b, $P > 0.05$).

S-9-PAHSA might partially activate the PI3K/AKT signaling pathway.

Western blot showed that the expression of PI3K, AKT, p-AKT and p-AKT/AKT ratio in T2DM group had a downward trend (Fig. 6e, $P < 0.05$; Fig. 6c, d, f, $P > 0.05$). After the intervention of S-9-PAHSA, the expression levels of both had an upregulated trend (Fig. 6c, F, $P > 0.05$). The results showed that S-9-PAHSA intervention might partially activate the PI3K/AKT signaling pathway in cells.

Discussion

PAHSAs has been reported to reduce blood glucose levels, enhance insulin sensitivity, and anti-inflammation in different diseases[3, 7, 8, 26, 27]. However, there was contradictory evidence showing that 5-PAHSA or 9-PAHSA isomers did not improve glucose control in mice[10]. These contrary studies may in large part due to the methodology chosen[28], but different PAHSAs isomers likely play a part as well. Therefore, we developed a new synthesis pathway separating the enantiomers of 9-PAHSA into S-9-PAHSA and R-9-PAHSA, and investigated their biological activities in SH-SY 5Y cells, and effect of S-9-PAHSA on glycolipid metabolisms in T2DM mice.

In this study, we found S-9-PAHSA could improve insulin resistance in HFD-induced T2DM mice, but it had no significant effect on fasting blood glucose, glycosylated serum protein and weight. Furthermore, the administration of S-9-PAHSA had no effect on release of GLP-1, an important hormone that stimulates insulin secretion[29, 30]. This implied that the anti-diabetic effect of S-9-PAHSA might not be exerted by reduction in glucose level, weight or directly GLP-1 secretion, while improvement in insulin resistance could be one possible reason for this phenomenon.

T2DM is mainly manifested insulin resistance. There is increasing evidence that insulin resistance is strongly induced by autophagy impairment. It was recently demonstrated that the role of insulin resistance and autophagy in some neurodegenerative diseases is crucial[31], and decreased autophagy could increase the risk of insulin resistance during the skeletal muscle aging process[32]. Impairment of autophagic flux in muscle cells induced insulin resistance directly[33]. Fujitani et al.[34] reported that reduced insulin secretion was closely related with the level of autophagy. In our studies, the effects of S-9-PAHSA on enhancing autophagy were revealed in SH-SY 5Y cells, and improvement of insulin resistance via elevating the activity on autophagy might be an important aspect of S-9-PAHSA's metabolic benefits, which is consistent with a previous study of PAHSA on hepatic and systemic insulin sensitivity[26].

Interestingly, we also found that the treatment of S-9-PAHSA could significantly inhibit the abnormal ox-LDL in the serum of HFD-induced T2DM mice, suggesting that S-9-PAHSA might have a promising effect on lipid metabolism disorders. Similarly, in our previous research, 5-PAHSA also exerted positive influence on lipid metabolism in DB/DB mice[35]. Many studies have verified that the stimulation of ox-LDL was related to suppression of autophagy. The enhancement autophagy could effectively reduce ox-LDL-induced RAW264.7 foam cell formation, reducing cellular lipid accumulation and delaying cell senescence[36]. Studies showed high glucose stimulated LDL transcytosis by a novel CAV1-CAVIN1-LC3B signaling-mediated autophagic degradation pathway[37]. Since T2DM patients usually suffered glucose metabolism disorders, low-density lipoprotein (LDL) could be saccharified by sustained hyperglycemia, then being swallowed by monocytes or macrophages, and causing intracellular cholesterol accumulation, and formatting foam cells that accelerate the development of atherosclerosis[38, 39]. At the same time, insulin resistance could cause abnormal activation of the lipid synthesis pathway, which in turn resulted in lipid deposition[40]. In addition, there is intense oxidative stress in lipid metabolism disorders. Recent studies have identified that the human umbilical vein endothelial cells exposed to ox-LDL exhibited enlarged ROS production[41]. Our study results demonstrated that the treatment of S-9-PAHSA could significantly decrease the level of ROS, suggesting that S-9-PAHSA might play a protective role through

anti-oxidative stress. Thus, targeting the crossed pathway between enhancement of autophagy and inhibition of ROS production may be a promising strategy with respect to the lipid metabolism after treatment of PAHSA.

Currently, autophagy, a metabolic pathway, has deserved widespread attention. It is beneficial to maintain the dynamic balance of cells[42]. There are a great deal of triggering factors, including ROS, LDL, inflammatory factors, etc, among which could cause the changes of autophagy regulation[43]. In our previous research, especially, it was confirmed that autophagy was involved in the development of T2DM-associated cerebrovascular disease and cognitive dysfunction[44, 45] and 9-PAHSA could promote autophagic flux in diabetic mice[6]. Previous reports demonstrated the suppression of high glucose on the activity of AMPK, which then consolidated BCL2-Beclin1, leading to autophagy inhibition in cardiomyocytes[46]. LC3II/I and Bcl-2, could signify the autophagic level. Specifically, when defective autophagy occurs, the expression of LC3II/I and Bcl-2 is down-regulated. In our experiments, through the stimulation of high glucose and lipid, the apparent decrease of LC3II/I and Bcl-2 were found, indicating that the autophagy level was decreased under the diabetic environment. However, this trend was reversed by the supplement of S-9-PAHSA, indicating that S-9-PAHSA might be helpful in preventing the glucose and lipids metabolism disorder via the alternation of autophagy.

Caspase family and Bcl-2 family proteins are key regulators in apoptosis[47, 48]. BAX and Bcl-2 are the major factors in the regulation of the mitochondria-mediated pathway of apoptosis. The ratio of BAX/Bcl-2 is a critical determinant of susceptibility to apoptosis, rather than the levels of individual proteins[49]. S-9-PAHSA might have an effect on apoptosis via caspase family, rather than Bcl-2 family, which was supported by our results that the levels of Cleaved caspase-3 were significantly decreased after S-9-PAHSA administration, but the ratio of BAX/Bcl-2 in GF + S-9-PAHSA group did not change compared with GF group.

This study showed that S-9-PAHSA increased protein expressions of PI3K and phosphorylation of AKT in SH-SY 5Y cells exposed diabetic environment, and this compound increased the level of autophagy, as well as reversed suppression on oxidative stress. The PI3K/AKT signaling pathway is a major mediator of insulin effects and plays a crucial role in T2DM pathogenesis. In patients with T2DM, alterations in the PI3K/AKT pathway primarily manifested as decreased phosphorylation[50]. Glucose transporter type 4 (GLUT4), mediated by the PI3K and AKT signaling pathways, also plays an important role in maintaining blood glucose homeostasis[51]. Numerous studies found that the level of autophagy was enhanced by the alternation of PI3K/AKT signal way[52, 53]. These results hinted that PI3K/Akt pathway was responsible for the protective actions of S-9-PAHSA in diabetic glycolipid metabolism dysfunction. However, further experiments are needed.

Conclusion

This study found that S-9-PAHSA could improve insulin resistance and lipid metabolism, and regulate autophagy-related proteins P62 in diabetic mice. At the same time, the study results also demonstrated

the S-9-PAHSA could alleviate cell's injuries under diabetic environment, promote cell proliferation, and reduce the levels of apoptosis by moderately regulating autophagy-related proteins and PI3K/Akt pathway. These results indicate that S-9-PAHSA may be a potent novel approach in treatment of T2DM by enhancing autophagy through upregulation of PI3K/AKT pathway.

Abbreviations

T2DM：Type-2 diabetes mellitus

PAHSA: Palmitic-Acid-Hydroxy-Stearic Acid

HFD: High-fat diet

STZ: Streptozotocin

FBG: Fasting blood glucose

Con: Control

Veh: Vehicle

HdS: High-dose S-9-PAHSA

LdS: Low-dose S-9-PAHSA

GSP: Glycosylated serum protein

GLP-1: Glucagon-like peptide-1

HOMA-IR: Homeostatic model assessment for insulin resistance

ox-LDL: Oxidized modified low-density lipoprotein

LDH: Lactate dehydrogenase

ROS: Reactive oxygen species

GSIS: Glucose-stimulated insulin secretion

GLUT4：Glucose transporter type 4

Declarations

Acknowledgements

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Contributions

Zhong-yu Yu, Jian-tao Wang and Jin-hong Lin draft the manuscript. Ying-chao Liu, Xiao-ming Zhang, Xiao-hong Wen and Qian Zhang carried out the statistical analysis, Xiao-die Xu, Cheng-feng He, Wen-jiao Xue, Xin-ru Wang, Jiao-qi Ren, Jin-xiu Wang and Yin Wang contributed discussion and edited manuscript. Hou-guang Zhou, Jing-chun Guo and Ji-chang Xiao revised manuscript. Hou-guang Zhou designed and supervised the project. The author(s) read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

All procedures were carried out according to the National Institutes of Health guide for the care and use of Laboratory animals and approved by animals ethical committee of Fudan University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data that supports the findings of this study are available from the corresponding author.

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Figures

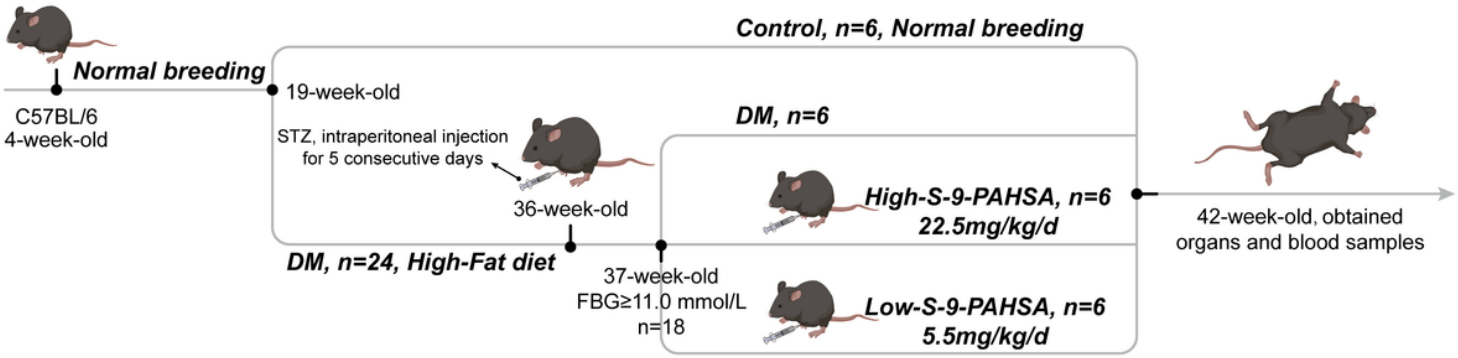

Figure 1

The experimental flow chart in mice

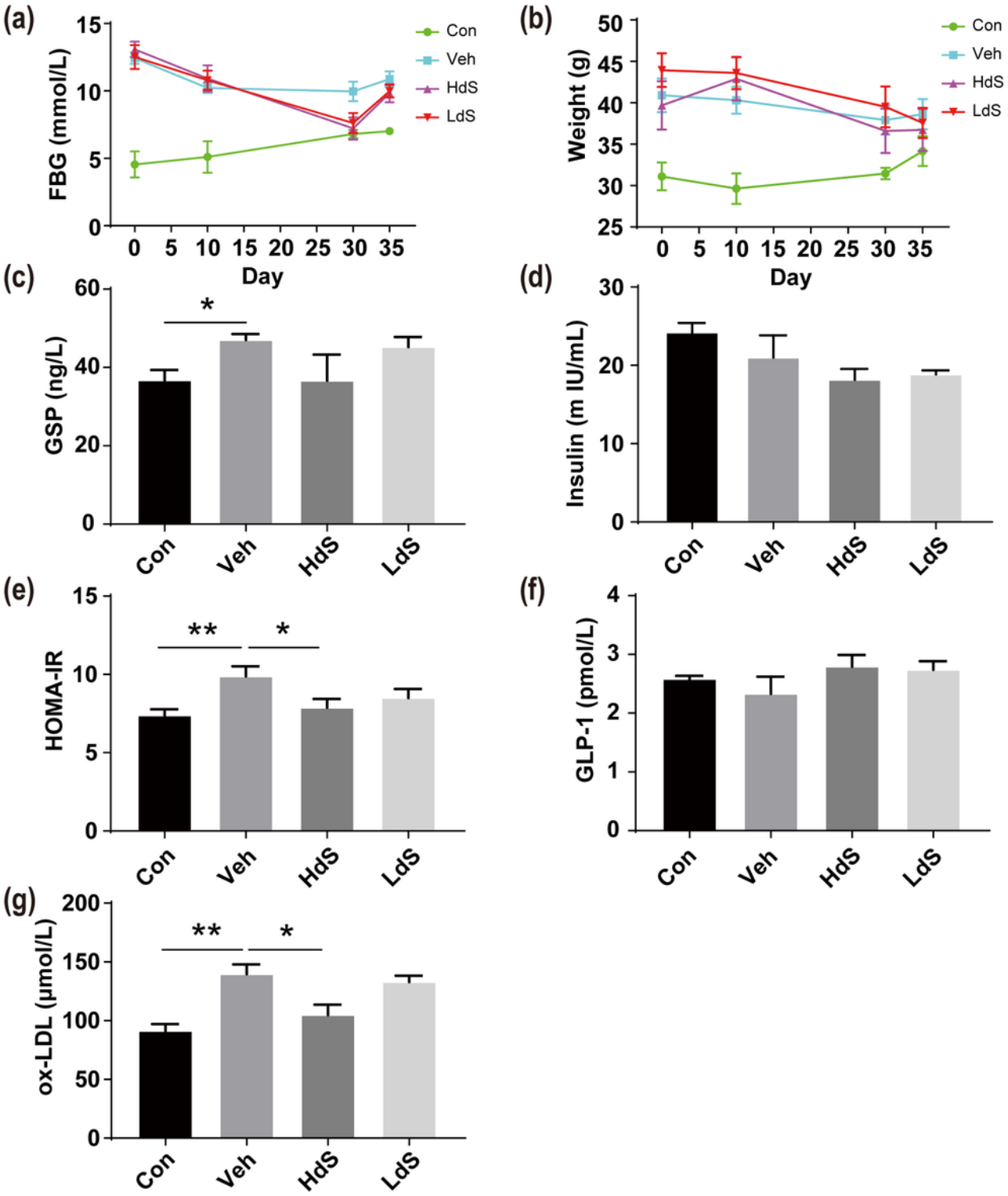

Figure 2

Effects of S-9-PAHSA on glucose and lipid metabolism in diabetic mice. (a-b) Fasting blood glucose and weight of C57BL/6 mice after S-9-PAHSA administration on days 10, 30, and 35. (c-f) GSP, insulin, HOMA-IR, GLP-1 and ox-LDL of diabetic mice after S-9-PAHSA administration. Data are mean ± SEM. n = 6 for a-f. *p < 0.05, **p < 0.01.

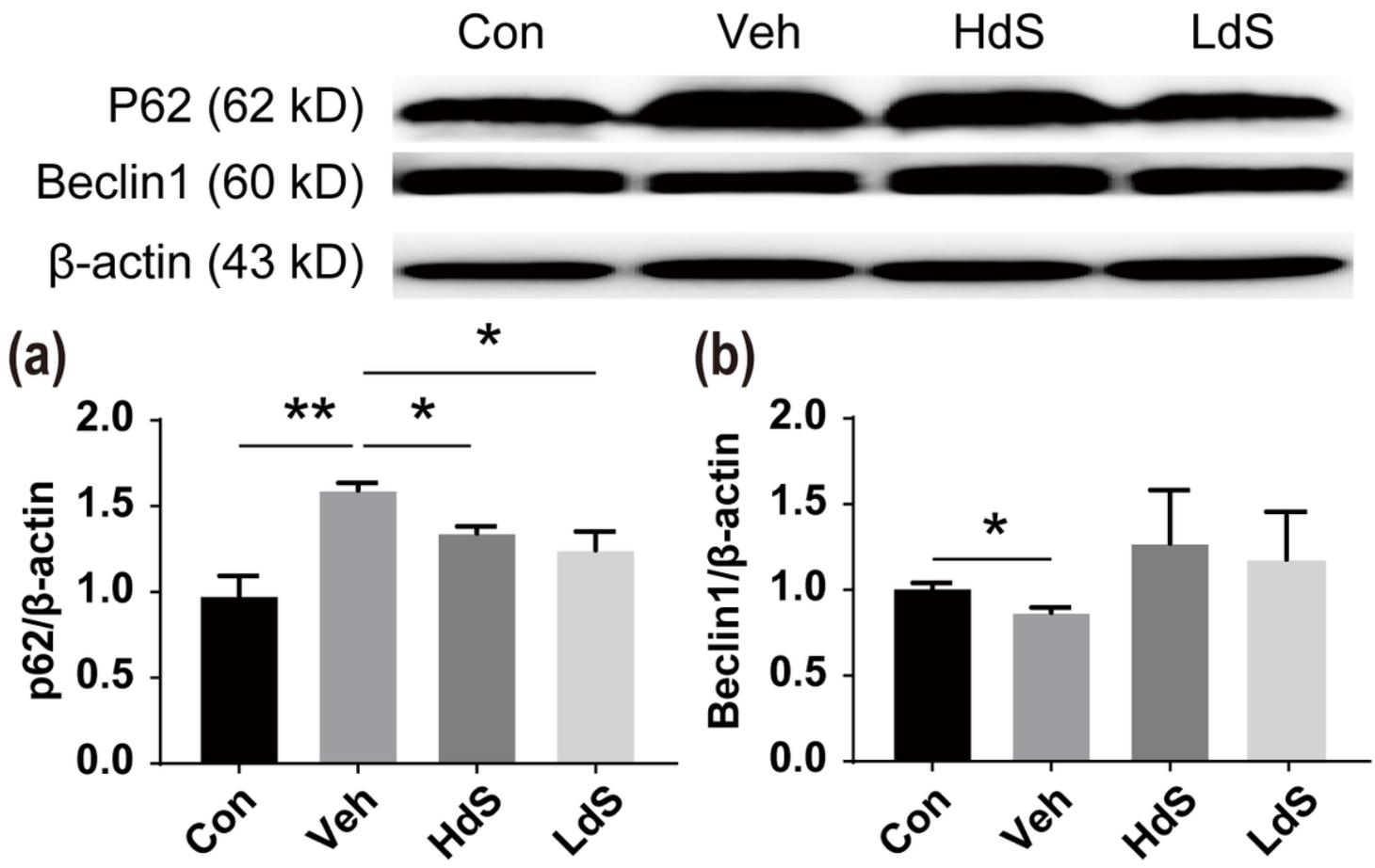

Figure 3

Effects of S-9-PAHSA on expression of autophagy protein in cerebral cortex of C57BL/6 mice. P62 (a), Beclin1 (b). Data are mean ± SEM. n = 3 for (a) and (b). *p < 0.05, **p < 0.01

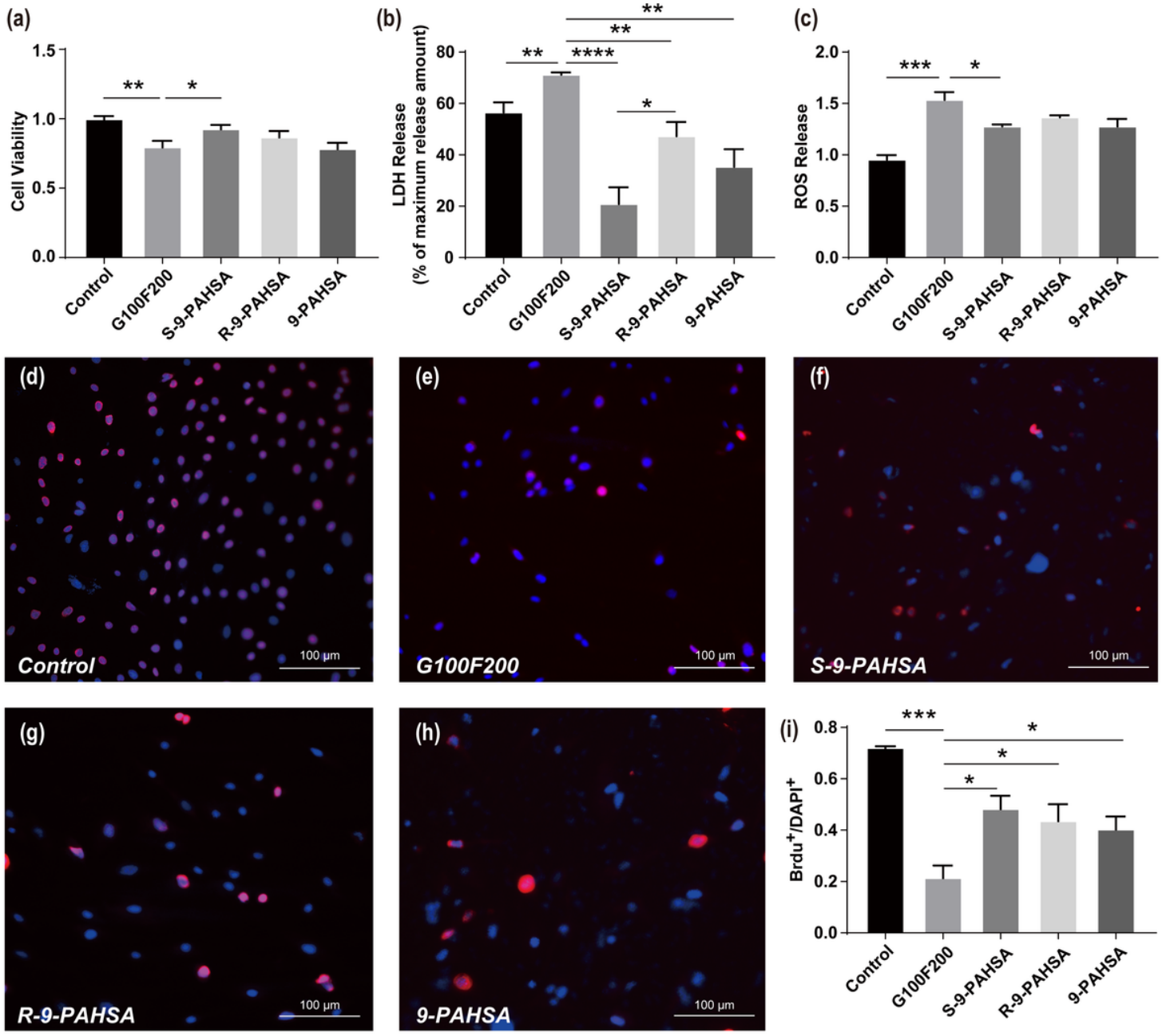

Figure 4

S-9-PAHSA alleviated diabetic environment-induced injury and reduced oxidative stress in SH-SY 5Y cells. Cell viability (a), LDH release (b), ROS release (c), BrdU Cell Proliferation Assay (d)-(i). Data are mean ± SEM. n = 4 for (a)-(c), n = 3 for (d)- (i). *p < 0.05, **p < 0.01, *** p < 0.001, **** p < 0.0001

Figure 5

Effects of S-9-PAHSA intervention on apoptosis levels in SHSY 5Y cells. Cleaved caspase-3 (a), BAX (b), Bcl-2 (c), BAX/Bcl-2 (d), TUNEL assay (e)-(h). Data are mean ± SEM. n = 4 for a-h. *p < 0.05, **p < 0.01

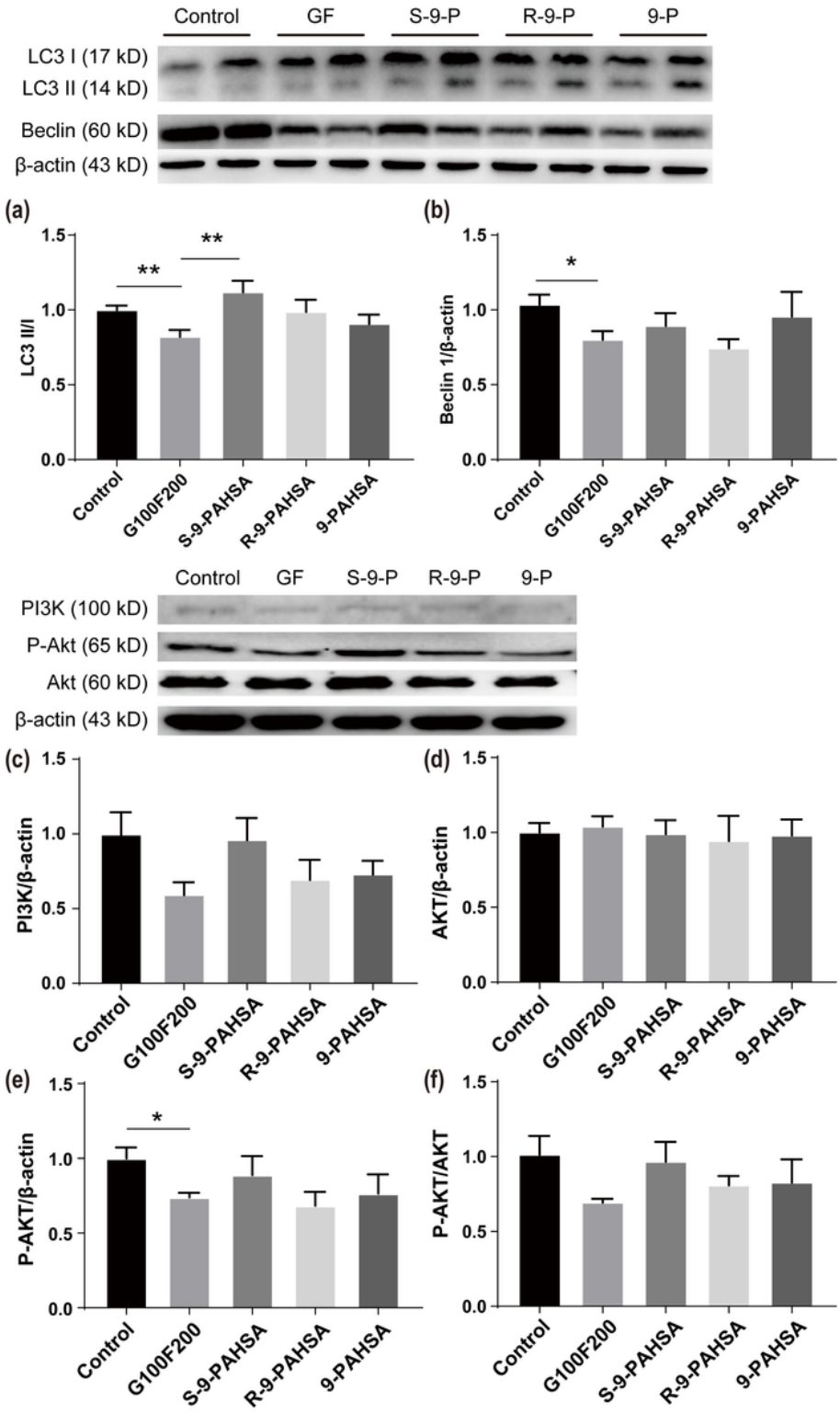

Figure 6

Effects of S-9-PAHSA on the PI3K/Akt signaling pathway in SH-SY 5Y cells. LC3 II/I (a), Beclin-1 (b), PI3K (c), AKT (d), p-AKT (e), p-AKT/AKT (f). Data are mean ± SEM. n = 3 for A-F. *p < 0.05, **p < 0.01

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