Original Article
Rapamycin protects against hepatic injury in streptozotocin-induced diabetic rats by enhancing autophagy

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Abstract: Type 2 diabetes mellitus (T2DM) increases the risk of liver disease and greatly accelerates the progression of the disease. The incidence of nonalcoholic fatty liver disease (NALFD) in diabetic patients is almost twice as high as that in healthy individuals. Unfortunately, effective therapies for hepatic injury caused by T2DM remain to be identified. A previous study reported that deletion of the autophagy-related Atg 5 or Atg 7 gene worsened hepatic injury. However, the association between liver injury and autophagy remains unclear. We used a streptozotocin (STZ)-induced diabetic rat model to investigate whether increased autophagy could alleviate liver injury caused by T2DM. Adult Sprague-Dawley (SD) rats were randomly assigned into one of four groups, namely, the normal control group (NC), diabetes mellitus group (DM), rapamycin-treated DM group (RAP), and hydroxychloroquine-treated DM group (HYD). We found that increased autophagy induced by RAP in diabetic rats could suppress hepatic injury caused by T2DM. RAP treatment also increased the body weight, decreased the blood glucose level, and alleviated some of the pathological changes associated with liver injury in diabetic rats. By contrast, decreased autophagy induced by HYD worsened liver injury. In conclusion, RAP-induced autophagy protected the liver in STZ-induced diabetic rats. Increasing autophagy through a pharmacological agent in diabetic subjects with hepatic injury may represent a feasible therapeutic approach.

Keywords: Autophagy, diabetes, hepatic, injury, LC3, rapamycin

Introduction
As estimated by the World Health Organization, type 2 diabetes mellitus (T2DM) is a major cause of death around the world [1]. The prevalence of nonalcoholic fatty liver disease (NALFD) is higher in patients with T2DM; therefore, there may exist a correlation between T2DM and NALFD [2]. NALFD is a pathological condition, characterized by excessive accumulation of triglycerides (TGs) within lipid droplets, which leads to defects in lipid metabolism [3, 4]. Increased free fatty acids (FFA) in the livers of diabetic patients contribute to steatosis, inflammatory steatohepatitis, cirrhosis, fibrosis, and even liver failure [5]. The mechanism behind hepatic injury caused by T2DM is unclear; however, recent studies have reported a connection between liver injury and autophagy.

Autophagy is a highly conserved cellular process that eliminates cytoplasmic components, such as damaged organelles within lysosomes, as well as aged proteins, and it is necessary for cell survival [6, 7]. A disruption in autophagy can cause various diseases, including neurodegeneration, cardiomyopathy, and cancer development [8, 9]. However, it is not known if abnormal autophagy can promote hepatic injury in diabetic patients.

Early evidence indicated that autophagy is involved in the degradation of intracellular lipids in hepatocytes [10]. Other studies reported that the knockdown of autophagy genes, namely, Atg 7 or Atg 5, or the use of autophagy inhibitors in mice fed with normal or high-energy diets (HD) increased the TG level in the liver. Similar results were obtained from experiments
Rapamycin protects against diabetic hepatic injury using cultured hepatocytes [11-15]. Furthermore, knockout of Atg 5 or Atg 7 in mice caused severe hepatocellular mitochondrial swelling and hepatomegaly [16-18], indicating that a suppression of autophagy may lead to an accumulation of TGs in the liver, and thus, severe hepatic injury.

There are two types of diabetes, namely, type 1 diabetes and type 2 diabetes, with T2DM being the most common type. At present, studies of T2DM mainly involve rat models, which may be due to the strong similarities in the disease between humans and rodents. The T2DM rat model requires that rats be fed with a HD, which is combined with a low dose of streptozotocin (STZ) [19]. Here, a HD and an injection of STZ were used to induce diabetes and to cause liver injury in rats. To induce or to inhibit autophagy, the rats were treated with rapamycin (RAP) or orhydroxychloroquine (HYD), respectively. RAP, a lipophilic macrolide antibiotic, inhibits the mammalian target of rapamycin (mTOR), thereby increasing autophagy [20]. On the other hand, HYD is a lysosomotropic compound that inhibits the degradation of autolysosomes, thereby increasing the lysosomal pH [21, 22]. Using this experimental model, we evaluated whether autophagy could affect the function of the liver and investigated the underlying mechanism.

Materials and methods

Experimental procedures and animals

Healthy adult male Sprague-Dawley (SD) rats (n = 80; initial body weight, 180-200 g) were acquired from the Laboratory Animal Center of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). The rats were acclimatized for 1 week, during which time they received a standard diet and fresh water ad libitum. The animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). This study was approved by the Institutional Animal Care and Use Committee of Huazhong University of Science and Technology.

Establishment of the diabetic rat model

The rats were randomly assigned into two groups. In the first group, namely, the normal control group (NC group), rats (n = 6) received a standard diet. In the second group (HD group), rats (n = 74) received a HD supplemented with 20% sucrose [w/w] and 20% lard [w/w]. After treatment for 4 weeks, the rats of the HD group received an injection of STZ (40 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.1 M citric acid buffer (pH 4.5) [23].

The rats of the NC group received an equivalent volume of citrate acid buffer (vehicle). The fasting blood glucose (FBG) level was measured after 3 days by applying a drop of tail blood to a portable glucometer (Changsha Sinocare Inc., China). Diabetic rats were defined as those with a FBG level > 11.1 mmol/L.

Healthy rats (i.e., NC group rats) received a standard diet. Diabetic rats were randomly divided into three groups (n = 6): those received vehicle (saline, 1.5 ml/day; i.e., DM group rats), rapamycin (RAP, 2 mg/kg daily p.o.; i.e., RAP group rats) or hydroxychloroquine (HYD, 60 mg/kg daily p.o.; i.e., HYD group rats) for 8 weeks [21].

Serum levels of biomarkers

The rats were treated for 8 weeks as indicated, fasted for 8 h, and then administered with anesthesia. To measure the FBG level, blood was collected from the abdominal aorta and centrifuged immediately. The serum levels of triglycerides (TGs), total cholesterol (TC), alanine aminotransferase (AST), and aspartate transaminase (ALT) were measured using an OLYMPUS AU2700 Chemistry Analyzer (Tokyo, Japan). The serum levels of interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) were measured using enzyme-linked immunosorbent assays (ELISA) (Nanjing Jiancheng Biotechnology Co., Ltd., Nanjing, China), according to the manufacturer’s instructions.

Histopathological analysis

After perfusion through the abdominal artery with ice-cold 0.8% (w/v) NaCl, the liver was harvested, minced into small fragments (size, 1 mm3), and fixed in either 2.5% (w/v) glutaraldehyde for electron microscopy or 10% (w/v) formaldehyde for histology. The remaining liver samples were stored at -80°C until use.

Hematoxylin and eosin staining

Paraffin sections (thickness, 5 μm) of the liver were stained with haematoxylin and eosin (HE) for histological analysis.
Masson staining

Paraffin sections (thickness, 5 μm) of the liver were stained using the Masson-Goldner Staining Kit. Fibrotic regions were stained blue, and hepatocytes were stained red.

Transmission electron microscopy

Liver tissues (size, 1 mm³) were fixed in 2.5% (w/v) glutaraldehyde, followed by 1% osmium tetroxide. The liver tissues were embedded in Epoxy Resin (618) and subsequently sectioned for examination under a Hitachi H-300 Transmission Electron Microscope (FEI Tecnai G2 Series, Holland).

Measurement of liver malondialdehyde (MDA) and superoxide dismutase (SOD) levels

The liver tissues were weighed and homogenized in 50 mM NaH₂PO₄ (pH 7.4) at a tissue:buffer ratio of 1:10. The MDA and SOD levels were measured using their respective kits (Nanjing Jiancheng Biotechnology Co., Ltd.) and a spectrophotometer.

Western blot analysis

The liver tissues were homogenized in 50 mM Tris, pH 7.5, containing 150 mM NaCl, 1% (v/v) Triton X-100, 1 mM phenylmethanesulfonyl fluoride, 1% (v/v) sodium deoxycholate, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 50 μg/ml leupeptin, and 50 μg/ml pepstatin. The lysates were incubated for 30 min at 4°C and then centrifuged for 30 min at 4°C. The protein concentration of the supernatants was measured using the Bicinchoninic Acid Assay (Beyotime, China). The proteins were separated by 15% SDS-PAGE and then transferred to polyvinylidenedifluoride membranes (Millipore, USA). The membranes were blocked in 5% (w/v) skimmed milk and washed with TBST, before incubating with primary antibodies against phosphorylated AMPK (P-AMPK), microtubule-associated protein 1 light chain 3 (LC3) (Abcam, Cambridge, MA, USA), mTOR (Sigma-Aldrich), Beclin-1 (Sigma-Aldrich) or GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) over night at 4°C. After washing three times with TBST, the membranes were incubated with horseradish peroxidase (HRP)-labeled secondary antibodies (Santa Cruz Biotechnology) at room temperature for 1 h. The ECL System was used to visualize the immune-active proteins, which were analyzed with the Kodak EDAS120 Imaging System (Tokyo, Japan).

Statistical analysis

All initial data were recorded using the excel database and statistical analysis were performed using SPSS 17.0 analytic software. The results were expressed as means ± SD. The optical density of every autophagic protein was measured by Quantity one software. All charts were graphed using GraphPad Prism 5.0 software. Comparisons between different experimental groups were carried out using one-way analysis of variance (ANOVA) with repeated measures, followed by Bonferroni post-hoc test. P-values < 0.05 were considered statistically significant.

Results

RAP improves various metabolic parameters

To investigate the correlation between liver injury and autophagy in diabetic rats, the body and liver weights, as well as the levels of FBG, TG, and TC, were obtained. The clinical symptoms of diabetes improved after autophagy was induced by RAP and worsened after autophagy was inhibited by HYD, as evidenced by the yellow coat and low energy of the rats in this group. Two rats in the HYD group died at the end of treatment; there was no death of rats in the other groups.

The metabolic characteristics are summarized in Table 1. The body weight of rats in the DM group was significantly lower ($P < 0.05$) than that of rats in the NC group, whereas the FBG level was significantly higher ($P < 0.05$). Furthermore, the liver-to-body weight ratio (LW/BW) of rats in the DM group was significantly higher than that of rats in the NC group ($P < 0.05$). The body weight of rats in the RAP group was significantly higher ($P < 0.05$) than that of rats in the DM group, whereas the FBG level and the HW/BW ratio in rats of the RAP group were lower ($P < 0.05$) than those in rats of the DM group. Compared to rats of the DM group, the body weight decreased, whereas the HW/BW and the FBG level increased in rats of the HYD group. The levels of TG and TC in fasting rats of the DM group were significantly higher ($P$
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Table 1. Effects of autophagy on metabolic abnormalities

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>LW/BW (%)</th>
<th>Fasting blood glucose (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>TC (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>458 ± 16.77</td>
<td>2.68 ± 0.25</td>
<td>4.1 ± 0.58</td>
<td>0.97 ± 0.09</td>
<td>1.28 ± 0.09</td>
</tr>
<tr>
<td>DM</td>
<td>255.17 ± 24.64*</td>
<td>5.15 ± 0.73*</td>
<td>16.9 ± 1.99*</td>
<td>1.21 ± 0.13*</td>
<td>1.52 ± 0.10*</td>
</tr>
<tr>
<td>DM + Rap</td>
<td>328.67 ± 23.13*</td>
<td>3.45 ± 0.57*</td>
<td>8.4 ± 2.44*</td>
<td>1.09 ± 0.14</td>
<td>1.37 ± 0.09*</td>
</tr>
<tr>
<td>DM + Hyd</td>
<td>232.00 ± 23.40*</td>
<td>5.55 ± 7.07*</td>
<td>17.4 ± 1.09*</td>
<td>1.26 ± 0.98*</td>
<td>1.49 ± 0.16*</td>
</tr>
</tbody>
</table>

*P < 0.05 compared to the NC group; "P < 0.05 compared to the DM control group. Data are presented as means ± SD (NC group, DM group and Rap group n = 6; Hyd group n = 4).

Figure 1. Changes in the body weight and the FBG level after the induction of autophagy. *P < 0.05 compared to the NC group; "P < 0.05 compared to the DM control group. Data are presented as means ± SD (NC group, DM group and Rap group n = 6; Hyd group n = 4).

< 0.05) than those in rats of the NC group. The TC level in rats of the RAP group was lower (P < 0.05) than that in rats of the DM group, whereas the TG level in rats of the RAP group was lower, although the results were not statistically significant.

The body weight and the FBG level of rats in all groups are presented in Figure 1 after the induction of autophagy by RAP. Compared to rats in the NC group, the body weight of rats in the DM group decreased significantly (P < 0.05). After treatment with RAP for 8 weeks, the body weight of rats in the RAP group increased significantly (P < 0.05) compared to rats of the DM group, except for the fourth week. Compared to rats in the DM group, however, the body weight of rats in the HYD group decreased.

The FBG levels in rats of DM and NC groups were approximately 12.50 mmol/L and 3.90 mmol/L, respectively. The FBG level decreased from 20.3 to 8.4 mmol/L after treatment with RAP for 8 weeks. The FBG level in rats of the HYD group was higher than that in rats of the DM group, although the results were not statistically significant. After treatment with RAP for 4 weeks, the FBG level in rats of the RAP group was lower (P < 0.05) than that in rats of the DM group.

RAP alleviates DM-induced hepatic injury, inflammation, and oxidative stress

To further investigate the correlation between liver injury and autophagy in diabetic rats, blood biochemical analysis was performed. Studies have reported that ALT mediates the conversion of alanine into glutamate and pyruvate in the liver, thereby serving as an excellent indicator of hepatic injury. The serum levels of AST and ALT in rats of the DM group were significantly higher (P < 0.05) than those in rats of the NC group (Figure 2A, 2B). The serum levels of ALT and AST in rats treated with RAP were lower (P < 0.05) than those in untreated rats. However, the serum levels of AST (P < 0.05) and
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Figure 2. RAP alleviates DM-induced hepatic injury, inflammation and oxidative stress. The levels of (A) AST, (B) ALT, (C) TNF-α, (D) IL-1β, (E) SOD, and (F) MDA; after 8 weeks of RAP treatment. *P < 0.05 compared to the NC group; #P < 0.05 compared to the DM control group. Data are presented as means ± SD (NC group, DM group and Rap group n = 6; Hyd group n = 4).

ALT in rats of the HYD group were higher than those in rats of the DM group.

The levels of TNF-α and IL-1β in rats of the DM group were significantly higher than those in rats of NC and RAP groups (Figure 2C, 2D). (P < 0.05). Furthermore, the SOD level in rats of the DM group was lower (Figure 2E) than that in rats of the NC group, whereas the MDA level was significantly higher (Figure 2F; P < 0.05), which was indicative of the accumulation of lipid peroxides. The SOD level was higher, and the MDA level was lower in rats of the RAP group than those in rats of the DM group (P < 0.05).

**RAP attenuates DM-induced hepatic injury**

The involvement of autophagy in hepatocyte dysfunction was examined by haematoxylin
Figure 3. RAP attenuates DM-induced hepatic injury. (A) Representative images of liver cross-sections stained with (A) Hematoxylin and eosin (magnification, 200’) or (B) Masson’s trichromestain (magnification, 200’). (C) Representative transmission electron micrographs of hepatocytes (magnification, 2,500’). I, NC group; II, DM group; III, RAP group; and IV, HYD group. The black arrow points to the autophagy in the liver cells.
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Figure 4. Changes in the levels of LC3, mTOR, P-AMPK and Beclin-1 after the induction of autophagy. A. LC3 II/I ratio in liver tissues from rats of NC, DM, RAP, and HYD groups, as detected by western blotting. B. mTOR protein level in liver tissues from rats of NC, DM, RAP, and HYD groups, as detected by western blotting and quantified by densitometry. C. Phosphorylated AMPK level in liver tissues from rats of NC, DM, RAP, and HYD groups, as detected by western blotting. D. Beclin-1 protein level in liver tissues from rats of NC, DM, RAP, and HYD groups, as detected by western blotting and quantified by densitometry. *P < 0.05 compared to the NC group; # P < 0.05 compared to the DM group. Data are presented as means ± SD (n = 3).

and eosin staining, Masson trichrome staining, and transmission electron microscopy (Figure 3). The rats in the NC group showed hepatic cells arranged in a cord-like manner, with intrahepatic lobules and clear hepatic sinusoidal structures. A round nucleus was located within each cell, and chromatin was uniformly distributed within the nucleus. In the DM group, vacuolar lesions showing spherical swelling were observed in hepatocytes, as well as a loose reticular cytoplasm and localized nuclear pyknosis. The RAP group showed mild swelling, and the normal arrangement of hepatocytes within cords. Most cells appeared normal. The ultrastructure of the liver in rats of the HYD group was worse than that of rats in the DM group. For instance, there were extensive vacuolar lesions within hepatocytes, and the structure of hepatic cord was unclear. Furthermore, there was evidence of local fat droplets, nuclear pyknosis, partial necrosis, and mild inflammatory cell infiltration. The results of the Masson staining in rat livers of the DM group showed that the number of collagen fibers staining blue was significantly higher than that in rat livers of the NC and RAP groups, whereas
the rats livers of the HYD group showed a deposition of collagen fibers that was similar to that of rat livers in the DM group.

The ubiquitin like kinase 1 (ULK1) complex plays an important role in the initiation of autophagy, and it serves as a bridge between upstream nutrient or energy receptor mTOR, AMPK and downstream autophagy [24]. Phosphorylated ULK1 has long been considered as a key regulator of autophagy. Recently, it has been reported that AMPK and mTOR can catalyze the phosphorylation of ULK1 and recruit ATGs, which lies downstream, to induce autophagy. Under physiological conditions, mTOR inhibits autophagy by inhibiting the activity of the ULK1 complex. Under stress or starvation, however, the activity of mTOR is inhibited and ULK1 complex is activated to induce autophagy. Several studies have reported that AMPK serves as a positive regulator of autophagy, mainly by inhibiting mTOR [25]. During autophagy nucleation, Beclin-1 can form complex with a variety of autophagy related molecules to promote autophagy [26-28]. LC3 also plays an important role [29, 30]. There are two isoforms of LC3 in cells. LC3-I is located in the cytoplasm, whereas LC3-II is located in the autophagic membrane. During the maturation of the autophagic membrane, cytoplasmic LC3-I is cleaved, exposing a carboxyl terminal glycine, which then localizes to the phagophore membrane to form LC3-II. The LC3-II level directly correlates with autophagosome abundance. So these proteins, mTOR, AMPK, Beclin-1 and LC3-II can be used as markers of autophagy level. Therefore, protein expressions of mTOR, AMPK, Beclin-1 and LC3 were measured by western blotting (Figure 4).

Our results revealed that the ratio of LC3 II/I (Figure 4A; \( P < 0.05 \)) and the level of mTOR (Figure 4B) in rats of the DM group were higher, whereas the P-AMPK (Figure 4C; \( P < 0.05 \)) and Beclin-1 (Figure 4D; \( P < 0.05 \)) levels in rats of the DM group were lower than that in rats of the NC group by western blot analysis. Compared to the DM group, the ratio of LC3 II/I and Beclin-1 (\( P < 0.05 \)) in rats of the RAP group were higher but were lower in rats of the HYD group. In addition, the mTOR level in rats of the RAP group was significantly lower (\( P < 0.05 \)) but significantly higher in rats of the HYD group (\( P < 0.05 \)) than that in rats of the DM group. The P-AMPK level in rats of the RAP group was significantly higher (\( P < 0.05 \)) than that in rats of the DM group, and lower in rats of the HYD group than that in rats of the DM group, although the results were without significant difference.

Discussion

NAFLD is the underlying cause of 75% of all chronic liver diseases, which associate with obesity and diabetes [31], and no effective therapeutic approach is available. A previous cohort study reported that T2DM contributes to the increased risk for liver disease. The incidence of NAFLD in diabetic patients is almost twice as high as that in healthy individuals. The typical pathological characteristics of NAFLD are inflammation, steatosis, cirrhosis, and fibrosis [32-34]. Autophagy is the adaptive response of cells to stress or injury. By autophagy, the cellular components (lipids, ribosomes, proteins, and even entire cell organelles) are degraded and reused [35]. The normal physiological function of hepatocytes depends on the basal autophagy level. The failure to eliminate potentially dangerous agents from the liver disturbs the homeostasis of the cells, thereby leading to severe hepatic diseases [36]. Numerous evidences indicate that the deletion of Atg 5 or Atg 7 causes the accumulation of subcellular organelles and proteins within hepatocytes, which results in an enlargement of the liver, serious injury to the liver, fibrosis, inflammation, tumorigenesis, and cirrhosis [16, 18, 37]. However, it is still unclear whether increased autophagy can protect against hepatic injury caused by T2DM.

In this study, the insulin resistance triggered by HD in rats was first investigated. Thereafter, a low dose STZ, which caused partial beta cell dysfunction and inhibited insulin secretion, served as a compensation for insulin resistance. After STZ was injected into rats fed with a HD for 4 weeks, the serum concentrations of glucose, TGs and TC increased, and the rats developed symptoms resembling those of type 2 diabetes. RAP or HYD, which can regulate autophagy in the livers of STZ-induced rats, affected autophagy, prompting us to propose a treatment for diabetic hepatopathy by applying RAP. The results indicate that increased autophagy prevented the induction of diabetes by hyperlipidemia and hyperglycemia. On the
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Contrary, decreased autophagy in diabetic rats had a worse effect. In conclusion, increased autophagy by RAP significantly increased the body weight, decreased the liver weight, and the blood glucose level. It also reversed some of pathological changes in the livers of diabetic rats. RAP might show favorable ameliorative effects on T2DM-induced NAFLD in rats, which is consistent with the results of He et al. [38], who reported that enhanced autophagy through Roux-en-Y gastric bypass exhibited hepatoprotective actions in STZ-induced diabetic rats. RAP is a typical mTOR inhibitor, which was first used as an antifungal agent, and was subsequently developed into an effective immunosuppressant and an antitumor drug. Along with prior findings on the decrease of hepatic autophagy associated with conditions that predispose rats to NAFLD, our findings suggest that increased autophagy induced by RAP can be clinically beneficial in T2DM-induced hepatic injury rat model.

The process of autophagy is complex; it can be divided into five closely connected stages, namely, autophagy activation, autophagy nucleation, autophagosome formation, autophagosome-lysosome fusion, cargo breakdown and release of degradation products. First, the mTOR kinase signaling pathway activates the intracellular ULK1 complex, followed by the recruitment of ATGs, which lies downstream, to induce autophagy [39]. After a series of complicated cascade reactions, autophagosomes are eventually formed. Autophagosomes fuse with lysosomes to form autolysosomes. After fusion, the inner vesicle along with its contents are degraded and then released into the cytosol.

In nutrient-rich conditions, mTOR combines signals from multiple upstream signaling pathways to negatively regulate the ULK1 complex [40, 41]. When mTOR is suppressed, either by a lack of nutrients or a pharmacological agent (e.g., RAP), the ULK1 complex is activated, which triggers its affinity for ATG13 and ATG17 to form a multi-protein complex. This results in the recruitment of other ATG proteins to form autophagosomes [42, 43]. Alternatively, mTOR inactivation may have activated autophagy via AMP-activated protein kinase (AMPK). AMPK is activated by upstream elements in instances of a low ATP level due to various causes. Activated AMPK, in turn, inhibits the activity of mTOR and consequently induces autophagy.

As shown in Figure 4, the LC3-II level was higher but the mTOR level was lower in rats of the RAP group than those of rats in the DM and HYD groups. These results show that RAP may induce autophagy by inhibiting the mTOR signaling pathway [20]. The level of P-AMPK in rats of the RAP group was higher than that in rats of the HYD and DM groups, which indicated that RAP may also positively regulate autophagy through AMPK-mediated inhibition of mTOR signaling [44]. Furthermore, the expression of Beclin-1 in RAP group was significantly higher than that in DM group and HYD group, indicating that RAP could also up-regulate autophagy by the Beclin-1 complex pathway.

We also found that autophagy was significantly increased in the rat livers of the DM group. A possible reason may be that the diabetic rat model induced by STZ, which is characterized by a loss of β-cells in the islets of the pancreas, resulted in hyperglycemia, followed by diabetes and diabetic complications. During this process, excessive free radical production and increased numbers of inflammatory cells caused an imbalance between oxidative and antioxidative systems. The increase in oxidative stress is implicated in the pathogenesis of diabetes and diabetic hepatopathy. Autophagy, as a stress adaptive pathway that avoids cell death, might have been activated by cellular injury factors such as oxidative stress and inflammatory cytokines. Indeed, the stress-induced autophagic clearance of protein aggregates and dysfunctional organelles is crucial for maintaining cellular homeostasis and normal physiological functions. Interestingly, the increased autophagy level could not offset the damage caused by STZ and HD in rats. Only by further increasing autophagy by RAP treatment can the damaged cell components be degraded, and autophagy can exert its protective effects on hepatocytes in diabetic subjects.

Conclusion

Our results indicate that RAP has hepatoprotective activity in STZ-induced diabetic rats by enhancing autophagy in hepatocytes. An up-regulation of autophagy in diabetic hepatopathy may be a target of clinical therapy for NAFLD induced by STZ. Clinical treatment of diabetic liver injury, such as treatment with an autophagy activator, may have a better therapeutic effect.
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Disclosure of conflict of interest

None.

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