Pharmacy Department[1], the First People's Hospital of Foshan, Foshan City, GuangDong Province; Medical School of China Three Gorges University[2], Yichang City, Hubei Province; Institute of Medicine[3], College of Medicine, Jishou University, Jishou City, Hunan Province; Cancer Research Institute[4], the First People's Hospital of Foshan, Foshan City, GuangDong Province, China

Chronic rapamycin treatment exacerbates metabolism and does not down-regulate mTORC2/Akt signaling in diabetic mice induced by high-fat diet and streptozotocin

YAN WANG[1], ZHI HE[2], XIANHUI LI[3], WENWEI CHEN[1], JIEWEN LU[1], XIANGPING CHEN[4], LIANG CAO[4]

Received November 4, 2014, accepted December 12, 2014

Yan Wang, Pharmacy Department, the First People's Hospital of Foshan, 81 Lingnan Road, Foshan 528000, Guangdong, China.
wangyantj2008@aliyun.com

Pharmazie 70: 604–609 (2015) *doi: 10.1691/ph.2015.4847*

Rapamycin, a classical inhibitor of the mammalian target of rapamycin (mTOR), has been intensively studied for its role in metabolism and verified to induce metabolic defects through mTORC2/Akt pathway. However, disparity of the results exists depending on the differences of the animal models or the detailed procedures. Moreover, data regarding the effect of rapamycin treatment in diabetic models are sparse. Therefore, we investigated its influence on glucose and lipid metabolism, and further analyzed its effect on the mTORC2/Akt pathway in a high-fat diet- and streptozotocin-induced diabetic mice model. Three-weeks old C57BL/6J mice were fed with a high fat diet (60 kcal% fat) and intraperitoneally injected with streptozotocin (100 mg/kg) at 6 weeks of age. Rapamycin (2 mg/kg) was orally given to the mice daily for consecutive 6 weeks. Body weight, blood lipid parameters and HbA_{1c}% values were evaluated. Oral glucose test and insulin tolerance test were performed. Furthermore, western blot assay was applied to investigate the protein epression levels of Akt and PKCα, two key targets of the mTORC2/Akt pathway. Rapamycin-treated diabetic mice demonstrated less weight gain, more profound symptoms of polydipsia, polyphagia and polyuria, significant liver fat accumulation and exacerbated metabolic disorders including insulin resistance, hyperglycemia and dyslipidemia. Contrary to what have been expected, though significantly inhibiting mTORC1/S6K1 signaling, chronic rapamycin treatment failed to down-regulate mTORC2/Akt pathway. Our findings provide evidence that chronic rapamycin treatment may exacerbate metabolism in diabetic subjects and does not down-regulate mTORC2/Akt signialing in a high-fat diet- and streptozotocin- induced diabetic mice model.

1. Introduction

Mammalian target of rapamycin (mTOR) is a highly conserved protein kinase which integrates a group of vital biological signals and acts as key regulator of cell growth and metabolism. mTOR exists in two distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Yang et al. 2007; Polak et al. 2009). Rapamycin, a classical mTOR inhibitor, is therefore widely used as immunosuppressor and anticancer agent for its strong anti-proliferative effect, and also used as an important research tool for mTOR signaling network.

Rapamycin was originally assumed to be solely responsive to mTORC1 signaling and beneficial for ameliorating insulin resistance partly due to its suppression of the mTORC1/S6K1 feedback loop (Um et al. 2004). Moreover, there is evidence demonstrating that rapamycin may effectively prevent the onset of type 1 diabetes for its potent immunoregulatory properties (Baeder et al. 1992; Jiang et al. 2009). However, more and more clinical studies (Johnston et al. 2008; Ciancio et al. 2012) as well as experimental research have paradoxically shown that rapamycin treatment inhibits pancreatic β cell proliferation, reduces glucose stimulated insulin release (Barlow et al. 2012) and induces insulin resistance by up-regulating hepatic gluconeogenesis and impairing lipid disposition in adipose tissue (Houde et al. 2010). The mechanism of the detrimental effect of rapamycin in glucose homeostasis has been attributed to its prevention of β-cells adaptation to hyperglycemia and inhibition of the mTORC1/S6K1 pathway (Fraenkel et al. 2008). Interestingly and importantly, some recent studies further revealed that mTORC2 is in fact sensitive to prolonged rapamycin treatment and the detrimental effect of rapamycin on metabolism is mediated at least in part *via* disrupting mTORC2 signaling (Barlow et al. 2012; Lamming et al. 2012; Ye et al. 2012).

Despite a plenty of studies characterizing the metabolic consequences of mTOR inhibition by rapamycin, disparity of the results exists depending on the differences of the animal models or the detailed procedures (dose, route, and frequency of rapamycin treatment). Moreover, due to the application of combination therapy, rapamycin's role in metabolism have significant confounding factors in most clinical investigations,

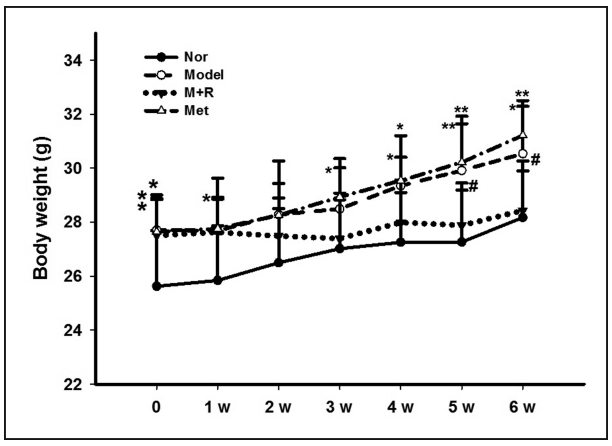

Fig. 1: Chronic rapamycin treatment induced significant less weight gain. Compared with normal mice, Model group had significant more body weight gain throughout the study period, whereas the M + R group showed strong tendency of less weight gain, and at the last two weeks of the study period, body weight of the M + R group were significantly lower than that of the Model group. Data are presented as mean ± S.E.M. for 12 mice each group. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. Nor group; $\#P < 0.05$ *vs*. Model group.

whereas research data regarding rapamycin administration in diabetic models have been sparse thus far. To address these, we applied a high-fat diet- and streptozotocin-induced diabetic mice model with and without pharmacologic mTOR inhibition by rapamycin, and we also studied the influence of chronic rapamycin administration on mTORC2/Akt signaling. Our study will provide further evidence for the optimized application of rapamycin in the clinical field.

2. Investigations and results

2.1. Body weight evolution

Over the 6 week course of the study, body weight of the diabetic mice increased over the matched normal mice (Fig. 1). However, rapamycin administration was associated with less weight gain that was statistically different from the Model group at the 5th and 6th week of the study.

2.2. Food and water consumption

As shown in Fig. 2A, the pad material of rapamycin-treated diabetic mice (left: red arrow) was more damp in comparison to that of the Model group (right: black arrow). Moreover, as shown in Figs. 2B and C, diabetic mice drank more water than normal mice, and chronic rapamycin treatment induced more profound symptoms of polydipsia and polyphagia, indicating deterioration of diabetes in M + R group.

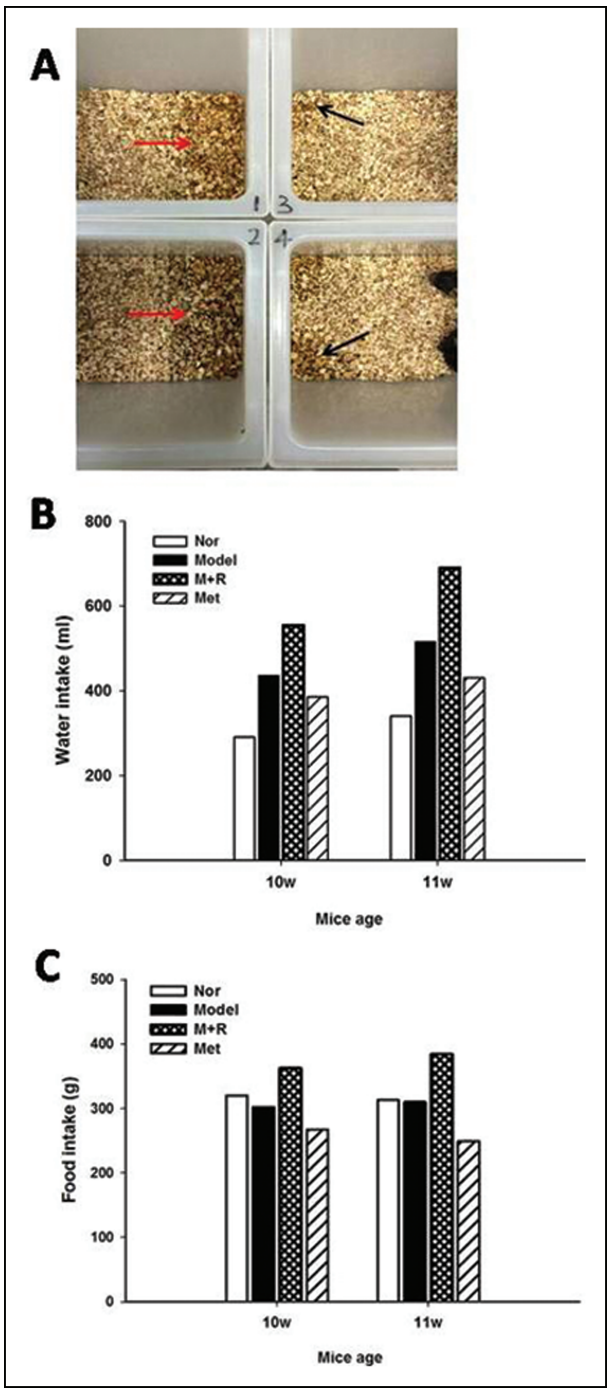

Fig. 2: Rapamycin-treated diabetic mice illustrated more profound symptoms of polydipsia, polyphagia and polyuria. (A) Pad material was more damp in the M + R group (left: red arrows) than in the Model group (right: black arrows). (B) and (C) At 10th and 11th week of age, food and water intakes of the mice were recorded daily, and the M + R group showed more profound symptoms of polyphagia and polydipsia than the Model group.

2.3. Oral glucose tolerance test

As shown in Fig. 3A, diabetic mice exhibited severely impaired glucose tolerance compared with normal mice, and AUC of the Model group was nearly three-fold that of the Nor group (Fig. 3B; $P < 0.01$). Rapamycin administration significantly increased blood glucose levels (Fig. 3A; $P < 0.05$) and OGTT AUC (Fig. 3B; $P < 0.01$) compared with the Model group, and correspondingly, mice in the M + R group demonstrated a strong tendency of significantly elevated HbA_{1C}% levels (12.86 ± 3.15; $P = 0.055$) in comparison to the model group (9.63 ± 1.36; Fig. 3C).

2.4. Insulin tolerance test

As demonstrated in Fig. 4A, in the insulin tolerance test, blood glucose concentration dropped much slower after insulin injection in the M + R group when compared with the Model group, the Nor group and the Met group, suggesting insulin sensitivity was further exacerbated in the M + R group. Moreover, as shown in Fig. 4B, diabetic mice exhibited significantly increased insulin levels, indicating the existence of insulin resistance (1.99 ± 1.03 ng/ml for the Model group and 0.87 ± 0.23 ng/ml for the Nor group; $P < 0.05$). Chronic rapamycin administration induced significantly decreased insulin levels in comparison to

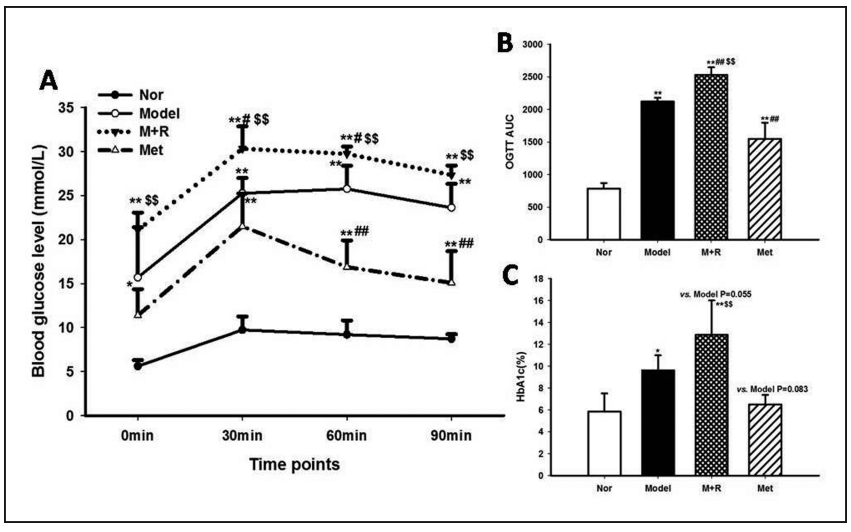

Fig. 3: Chronic rapamycin treatment exacerbated glucose metabolism. (A) Rapamycin treatment induced significant exacerbation of glucose metabolism in OGTT compared to Nor group, Model group and Met group. (B) Rapamycin-treated diabetic mice demonstrated significantly increased OGTT AUC in contrast to Nor group, Model group and Met group. (C) M + R group showed strong tendency of increased HbA_{1c}% level when compared with the Model group (P = 0.055). Data are presented as mean ± S.E.M. for 6 mice each group. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs*. Nor group; #$P < 0.05$, ## $p < 0.01$ *vs*. Model group; \$\$$p < 0.01$ *vs*. Met group.

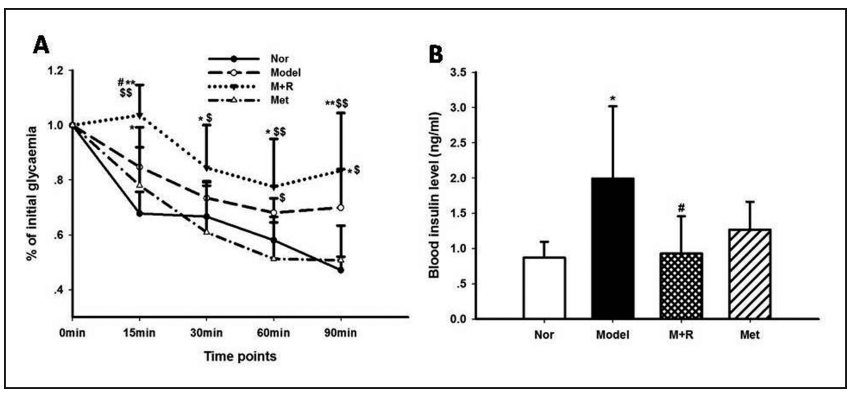

Fig. 4: Rapamycin treatment exacerbated peripheral insulin resistance. (A) In the insulin tolerance test, blood glucose values dropped much slower after insulin injection in M + R group compared to Nor group, Model group and Met group. (B) Model mice demonstrated significantly increased blood insulin level, and rapamycin treatment reduced blood insulin level when compared with the Model group. Data are presented as mean ± S.E.M. for 6 mice each group. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs*. Nor group; #$P < 0.05$, ## $p < 0.01$ *vs*. Model group; \$$p < 0.05$, \$\$$p < 0.01$ *vs*. Met group.

the Model group (1.99 ± 1.03 ng/ml for the Model group and 0.93 ± 0.53 ng/ml for the M + R group; $P < 0.05$).

2.5. Lipid metabolism

As shown in Figs. 5(1) and 5(2), obvious hepatic steatosis was observed in the Model group (B) and the M + R group (C) when compared with the Nor group (A). Correspondingly, as shown in Figs. 5(3A), 5(3B) and 5(3C), blood lipid levels were significantly increased in the Model group, the M + R group and the Met group in comparison to the Nor group. Furthermore, as demonstrated in Figs. 5(3D) and 5(3E), liver total triglyceride content (3.99 ± 0.48 mmol/gprot for the M + R group and 3.07 ± 0.51 mmol/gprot for the Nor group) and liver total cholesterol content (3.19 ± 0.57 mmol/gprot for M + R group the and 2.25 ± 0.47 mmol/gprot for the Nor group) were significantly elevated in the M + R group compared with the Nor group, whereas liver fat content between the Model group and the Nor group did not reach statistical significance, suggesting exacerbation of lipid metabolism in the M + R group.

2.6. WB analysis

As shown in Fig. 6B, the M + R group demonstrated significantly down-regulated phosphorylation of S6K1-T389 in liver tissue of the mice, suggesting inhibition of the mTORC1 pathway by chronic rapamycin administration. However, rapamycin treatment did not down-regulate phosphorylation of Akt-S473 and PKCα-Ser 657 when compared with Model group, as shown in Figs. 6C and D.

3. Discussion

In the present study, we showed for the first time that in a high-fat diet- and streptozotocin- induced diabetic mice model, chronic rapamycin treatment exacerbated metabolic defects including insulin resistance, hyperglycemia and dyslipidemia.

Rapamycin, a specific inhibitor of the mTORC1 pathway, is frequently used as immunosuppressant and anticancer agent in both transplantation and oncology. Nevertheless, growing evidence has suggested that rapamycin is associated with new-onset diabetes in a number of clinical studies and experimental research (Barlow et al. 2013).

Consistent with previous studies, we found that rapamycin treatment significantly impaired glucose tolerance, increased HbA_{1c}% values and exacerbated insulin resistance compared with the diabetic control mice. Glucose homeostasis relies on two main facts: intact insulin-producing capacity of pancreatic β-cells and insulin sensitivity of peripheral organs. Rapamycin was reported to reduce β-cell function and mass,

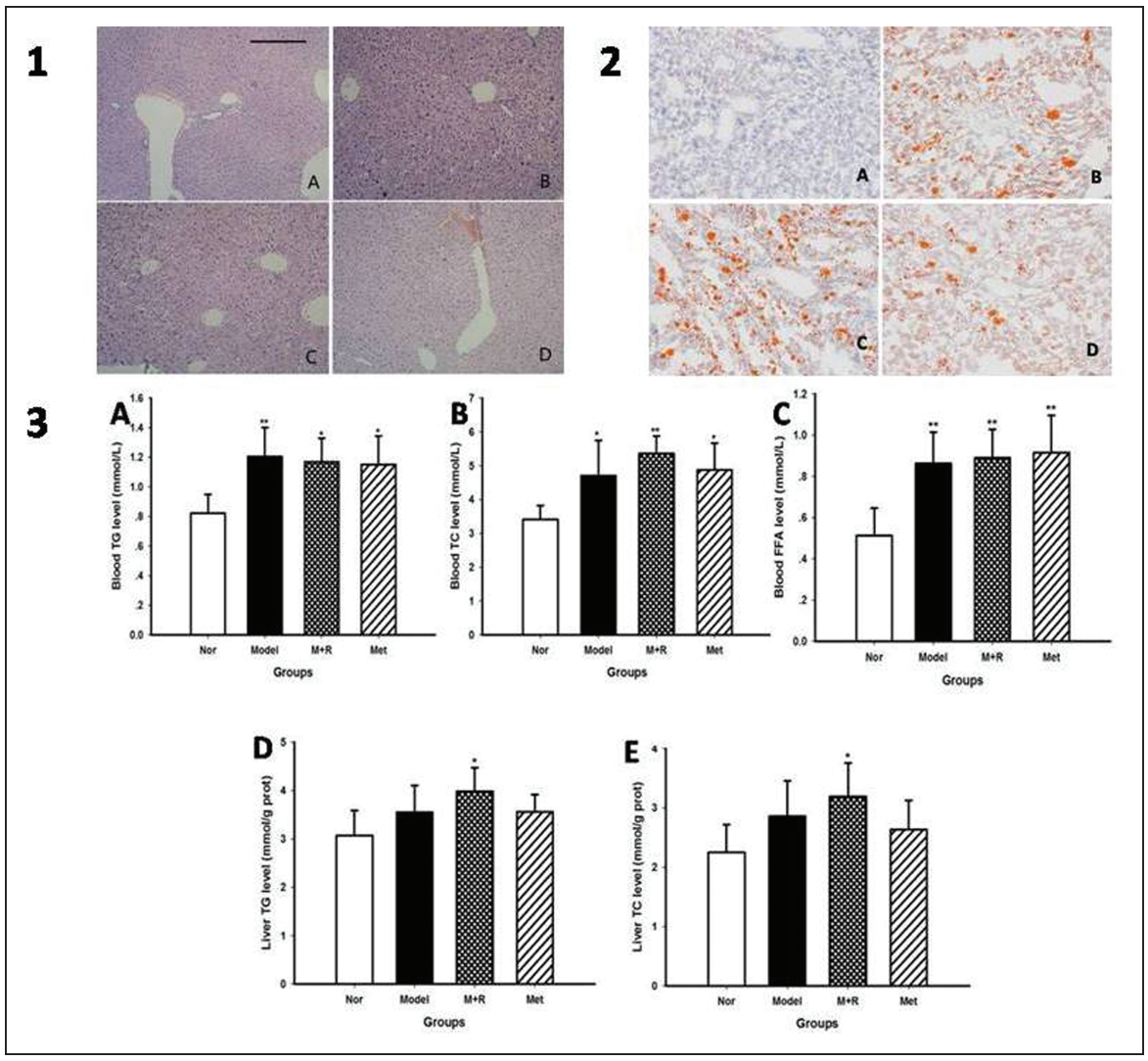

Fig. 5: Chronic rapamycin treatment exacerbated lipid metabolism. (1) HE staining of the liver tissue (×200). (2) Oil red staining slides of the liver tissue (×200). Significant hepatic steatosis was observed in the Model group (B) and the M + R group (C) when compared with the Nor group (A) (3) (A) Diabetic mice demonstrated significantly increased blood TG level compared with the Nor group. (B) Diabetic mice demonstrated significantly increased blood TC level compared with the Nor group, and the M + R group illustrated tendency of elevated TC level in contrast to the Model group and the Met group. (C) Diabetic mice demonstrated significantly increased FFA levels compared with the Nor group. (D) Rapamycin treatment significantly increased liver TG content when compared with the Nor group. (E) Rapamycin treatment significantly increased liver TC content compared with the Nor group. Data are presented as mean ± S.E.M. for 6 mice each group. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs*. Nor group.

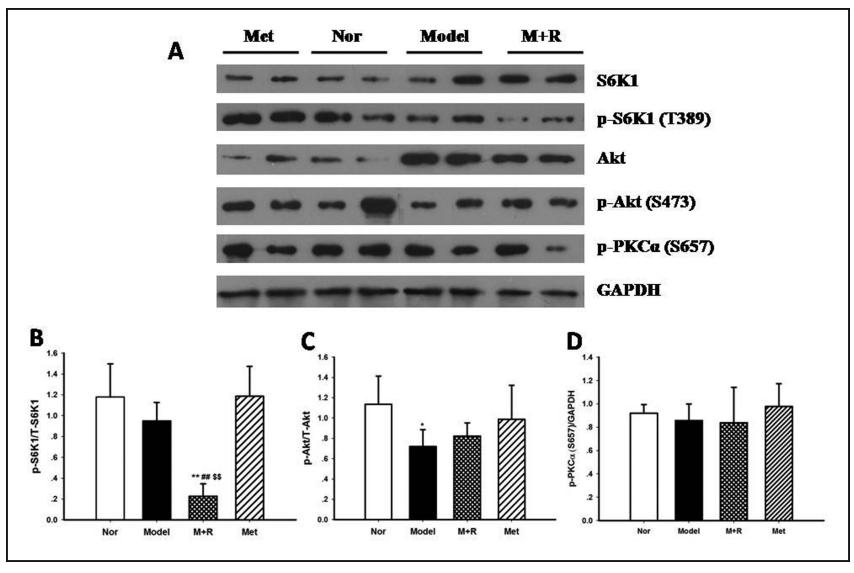

Fig. 6: Rapamycin significantly inhibited mTORC1/S6K1 signaling, whereas exerted no influence on mTORC2/Akt pathway. (A) Western blot bands of the four groups. (B) Chronic rapamycin administration significantly inhibited phosphorylation of S6K1 (T389) in liver tissue of the mice, suggesting inhibition of mTORC1 pathway. (C) and (D) Rapamycin treatment did not down-regulate phosphorylation of Akt-S473 and PKCα-Ser 657 when compared with the Model group. Data are presented as mean ± S.E.M. for 6 mice each group. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs*. Nor group; ## $p < 0.01$ *vs*. Model group; $$$p < 0.01$ *vs*. Met group.

damage insulin-producing capacity of β-cells, thus significantly decreased serum insulin level in the *Psammomys obesus* model of nutrition-dependent type 2 diabetes (Fraenkel et al. 2008). Furthermore, fasted insulin level was decreased after weekly administration of rapamycin in a high-fat diet-fed mice model (Makki et al. 2014). Nevertheless, 21 days of rapamycin treatment has been verified to induce significant insulin resistance, by attenuating glucose uptake and metabolism in skeletal muscle, and increase blood insulin level in rats fed with a standard diet (Deblon et al. 2012). Moreover, another study convinced that 15 days of rapamycin administration led to significant insulinaemia in Male Sprague-Dawley rats (Houde et al. 2010). The difference of the results may lie in several aspects, one of which being the actual state of β-cells (capacity to proliferate and differentiate) before initiation of rapamycin treatment. In the present research, high-fat diet in combination with streptozotocin injection induces a typical type 2 diabetic mice model (Wu et al. 2009). Though damaged by streptozotocin and chronic high-fat diet feeding, pancreatic β-cells of the diabetic animals still experience a rapid, though limited, increase of proliferation to compensate for the glucose challenge. However, due to its strong anti-proliferative effect, rapamycin treatment abolished this compensation, and rapamycin-treated diabetic mice therefore had significantly decreased blood insulin levels compared with their diabetic counterparts.

Dyslipidemia is often concurrent with hyperglycemia due to the close relationship between glucose and lipid metabolism (Wang et al. 2011). Activation of mTORC1 signaling has been demonstrated to suppress lipolysis, increase de novo lipogenesis and promote intracellular accumulation of triglycerides (Chakrabarti et al. 2010), therefore mTORC1 inhibition by rapamycin was able to significantly increase blood TG and FFA levels (Fraenkel et al. 2008; Houde et al. 2010), decrease lipogenesis, inhibit adipocyte differentiation and reduce fat mass of high-fat diet fed C57BL/6J mice (Makki et al. 2014; Ray et al. 2014). In the present study, we consistently found that rapamycin administration induced less weight gain of the diabetic animals. Furthermore, rapamycin treatment significantly increased liver fat content of the diabetic animals. Physiologically, adipose tissue is the main "storehouse" of lipid. However, rapamycin treatment significantly decreases fat cell size and fat mass, which contributes to the elimination of the "storehouse". The excessive fat taken in by rapamycin-treated diabetic mice, due to the profound symptom of polyphagia, have therefore "nowhere to go" but accumulate in the liver tissue, leading to exacerbation of lipid metabolism.

mTORC1 has been confirmed to play an important role in the regulation of β-cell mass. Hyperactivation of mTORC1 leads to increases in β-cell size and mass, leading to improvements in insulin secretion and glucose tolerance (Hamada et al. 2009). Furthermore, mice deficient in S6K, one of the best characterized targets of mTORC1, are also hypoinsulinemic and glucose intolerant with diminished β-cell size (Pende et al. 2000). Rapamycin, the specific mTORC1 inhibitor, is therefore presumed to induce diabetes by inhibition of mTORC1/S6K1 signaling (Fraenkel et al. 2008). Nevertheless, prolonged rapamycin treatment was demonstrated capable of affecting mTORC2 signaling (Sarbassov et al. 2006; Barlow et al. 2012), and recent studies revealed that mTORC2 activity played a leading role in both β-cell survival and function (Gu et al. 2011). Therefore, rapamycin β-cell toxicity may also be mediated by inhibition of mTORC2 signaling. Moreover, in addition to the direct effect of rapamycin on β-cells described above, further evidence suggests that rapamycin-induced insulin resistance is mediated predominately *via* mTORC2 rather than mTORC1 (Lamming et al. 2012). However, weekly administration of rapamycin treatment for 22 weeks led to beneficial metabolic effects through mTORC1 inhibition whilst mTORC2 activity was intact (Makki et al. 2014). In the present study, chronic rapamycin administration was proved to significantly down-regulate mTORC1/S6K1 signaling, whereas relative expression of p-Akt (S473) and p-PKCα (S657), two identified downstream effectors of mTORC2 pathway, were not altered compared with the Model group. These results suggested that treatment with rapamycin (2 mg/kg/d) for 6 weeks did not block mTORC2 signaling, partly because expression of p-Akt (S473) and p-PKCα (S657) was either significantly down-regulated or unchanged in the Model group compared to Nor group.

In conclusion, we demonstrated that chronic rapamycin treatment exacerbates glucose and lipid metabolism, whereas does not down-regulate mTORC2/Akt signaling in the high-fat diet- and streptozotocin- induced diabetic mice model. Our findings provide an important theoretical background for clinical application of rapamycin treatment of diabetic patients.

4. Experimental

4.1. Animal model

Three week old male C57BL-6J mice were fed on high fat diet containing 60 kcal% fat. At 6 weeks of age, mice were injected with streptozotocin (100 mg/kg; Sigma Chemical Co., USA) intraperitoneally. C57BL-6J mice and high fat diet were both bought from Guangdong Medical Laboratory Animal Center (Guangzhou, Guangdong Province, China). At 9 weeks of age, diabetic mice were selected, and those with similar blood glucose levels were included in the study. Mice were assigned to four groups (n = 12): (1) Nor group (normal mice), (2) Model group (diabetic mice: high fat diet + streptozotocin), (3) M + R group (Rapamycin-treated diabetic mice), and (4) Met group (metformin-treated diabetic mice).

Rapamycin (2 mg/kg body weight; LC Laboratories, Woburn, MA, USA) was diluted in a vehicle solution (sterile 10% PEG400/8% ethanol, followed by an equal volume of sterile 10% Tween 80) and given to the mice daily by intragastric administration for consecutive 6 weeks. Metformin (300 mg/kg; Sigma Chemical Co., USA) was diluted in the drinking water daily. The mice in Nor group and Model group were administered the same volume of solvent.

Mice had free access to the drinking water and were maintained in a temperature- and humidity-regulated room (22 ± 2 °C, 55 ± 15% RH) with controlled lighting (12-h light/dark cycle, 8:00 a.m. to 8:00 p.m.).

All animal care and experimental procedures complied with the guidelines for the Care and Use of Laboratory Animals, formulated by the Ministry of Science and Technology of the People's Republic of China, were approved by the Ethical Committee on Animal Experiments at the First People's Hospital of Foshan (approval number 141255).

4.2. Body weight evolution and water and food intake

Body weight was monitored every week. Food and water intake of the mice was measured daily by substracting the remnant from the total amount at 10^{th} and 11^{th} week of age. Since there was the same number of mice in each group, food and water intakes were compared as the total amount consumed by the 12 mice altogether.

4.3. Insulin tolerance test and glucose tolerance test

Mice were fasted for 4 h and then injected with insulin (0.5 IU/kg; NovoDisk, Norvartis, Denmark) intraperitoneally. Blood glucose levels were measured at 0 min, 15 min, 30 min, 60 min and 90 min.

An oral glucose test was performed at the end of the study. Mice were fasted for 8 h, and glucose was administered orally to the mice at a dose of 2.5 mg/kg body weight. Blood glucose levels were measured at 0 min, 30 min, 60 min and 90 min.

Blood glucose values were determined with a portable glucose meter (Roche, Switzerland). The results of the oral glucose tests were expressed as integrated area under the curve (AUC) for glucose concentrations, and the results of insulin tolerance test were expressed as % of initial glucose values at 0 min of the test.

4.4. Blood biochemical parameters

At the end of the study, mice were fasted for 8 h followed by 3 h of refeeding to achieve physiological activation of insulin signaling, after which mice were decapitated to get the blood samples. Liver tissues were excised, rinsed with chilled sterile saline, frozen in liquid nitrogen immediately and stored

along with blood samples at − 80 °C. For pathological studies, liver tissues were immersed in 4% formalin for later analysis.
Plasma triglyceride, total cholesterol and nonesterified fatty acid levels in addition to liver triglyceride and total cholesterol values were determined using commercial kits (Beijing Strong Biotechnologies Inc, China). Plasma insulin and HbA_{1c}% concentrations were measured using Millipore Elisa kit and Kamiya Elisa kit, respectively.

4.5. Histopathological studies

Liver HE staining was performed on consecutive sections (5 μm) of paraffin-embedded liver tissue. For Oil red O staining, liver sections were fixed in 4% buffered formaldehyde for 5 min at room temperature. Staining of intracellular neutral lipids was performed with Oil red O and sections were further stained with Harris' Hematoxylin.

4.6. Western Blot analysis

Liver samples were homogenised and lysed on ice in RIPA buffer. Homogenates were rotated for 40 min and centrifuged (12,000 g for 10 min at 4 °C). Protein of total lysate (30 μg) was subjected to SDS-PAGE and blotted on to nitrocellulose membrane. Primary antibodies against S6K1, Phospho-S6K1 (Thr 389), Akt, Phospho-Akt (Ser 473) were purchased from Cell Signaling Technology (Beverly, MA, USA) and Phospho-PKCα (Ser 657) antibody was bought from Santa Cruz Biotechnology (CA, USA). The proteins were detected using horseradish peroxidase-conjugated anti-rabbit or anti-goat secondary antibodies. The optical density values of bands were measured with Image J software.

4.7. Statistical analysis

Data are shown as means ± SE. One-way analysis of variance (ANOVA) was used to test the homogeneity for variance and Tukey's test was applied to test the significance of differences between multiple groups. A P value of less than 0.05 was considered significant.

Acknowledgements: This project was supported by the grant from the National Natural Science Foundation of China (Grant No. 81302824).

References

Baeder WL, Sredy J, Sehgal SN, Chang JY, Adams LM (1992) Rapamycin prevents the onset of insulin-dependent diabetes mellitus (IDDM) in NOD mice. Clin Exp Immunol 89: 174–178.

Barlow AD, Xie J, Moore CE, Campbell SC, Shaw JA, Nicholson ML, Herbert TP (2012) Rapamycin toxicity in MIN6 cells and rat and human islets is mediated by the inhibition of mTOR complex 2 (mTORC2). Diabetologia 55: 1355–1365.

Barlow AD, Nicholson ML, Herbert TP (2013) Evidence for rapamycin toxicity in pancreatic β-cells and a review of the underlying molecular mechanisms. Diabetes 62: 2674–2682.

Chakrabarti P, English T, Shi J, Smas CM, Kandror KV (2010) Mammalian target of rapamycin complex 1 suppresses lipolysis, stimulates lipogenesis, and promotes fat storage. Diabetes 59: 775–781.

Ciancio G, Sageshima J, Chen L, Gaynor JJ, Hanson L, Tueros L, Montenora-Velarde E, Gomez C, Kupin W, Guerra G, Mattiazzi A, Fornoni A, Pugliese A, Roth D, Wolf M, Burke GW 3rd (2012) Advantage of rapamycin over mycophenolate mofetil when used with tacrolimus for simultaneous pancreas kidney transplants: randomized, single-center trial at 10 years. Am J Transplant 12: 3363–3376.

Deblon N, Bourgoin L, Veyrat-Durebex C, Peyrou M, Vinciguerra M, Caillon A, Maeder C, Fournier M, Montet X, Rohner-Jeanrenaud F, Foti M (2012) Chronic mTOR inhibition by rapamycin induces muscle insulin resistance despite weight loss in rats. Br J Pharmacol 165: 2325–2340.

Fraenkel M, Ketzinel-Gilad M, Ariav Y, Pappo O, Karaca M, Castel J, Berthault MF, Magnan C, Cerasi E, Kaiser N, Leibowitz G (2008) mTOR inhibition by rapamycin prevents beta-cell adaptation to hyperglycemia and exacerbates the metabolic state in type 2 diabetes. Diabetes 57: 945–957.

Gu Y, Lindner J, Kumar A, Yuan W, Magnuson MA (2011) Rictor/mTORC2 is essential for maintaining a balance between beta-cell proliferation and cell size. Diabetes 60: 827–837.

Houde VP, Brûlé S, Festuccia WT, Blanchard PG, Bellmann K, Deshaies Y, Marette A (2010) Chronic rapamycin treatment causes glucose intolerance and hyperlipidemia by upregulating hepatic gluconeogenesis and impairing lipid deposition in adipose tissue. Diabetes 59: 1338–1348.

Hamada S, Hara K, Hamada T, Yasuda H, Moriyama H, Nakayama R, Nagata M, Yokono K (2009) Upregulation of the mammalian target of rapamycin complex 1 pathway by Ras homolog enriched in brain in pancreatic beta-cells leads to increased beta-cell mass and prevention of hyperglycemia. Diabetes 58: 1321–1332.

Jiang GX, Cui YF, Zhong XY, Tai S, Liu W, Wang ZD, Shi YG (2009) Use of a cocktail regimen consisting of soluble galectin-1, rapamycin and histone deacetylase inhibitor may effectively prevent type 1 diabetes. Arch Med Res 40: 424–426.

Johnston O, Rose CL, Webster AC, Gill JS (2008) Sirolimus is associated with new-onset diabetes in kidney transplant recipients. J Am Soc Nephrol 19: 1411–1418.

Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, Davis JG, Salmon AB, Richardson A, Ahima RS, Guertin DA, Sabatini DM, Baur JA (2012) Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science 335: 1638–1643.

Makki K, Taront S, Molendi-Coste O, Bouchaert E, Neve B, Eury E, Lobbens S, Labalette M, Duez H, Staels B, Dombrowicz D, Froguel P, Wolowczuk I (2014) Beneficial metabolic effects of rapamycin are associated with enhanced regulatory cells in diet-induced obese mice. PLoS One 9: e92684.

Polak P, Hall MN (2009) mTOR and the control of whole body metabolism. Curr Opin Cell Biol 21: 209–218.

Pende M, Kozma SC, Jaquet M, Oorschot V, Burcelin R, Le Marchand-Brustel Y, Klumperman J, Thorens B, Thomas G (2000) Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. Nature 408: 994–997.

Ray PD, Maclellan RA, He J, Liu Z, Wu J (2014) Downregulation of RelA (p65) by Rapamycin Inhibits Murine Adipocyte Differentiation and Reduces Fat Mass of C57BL/6J Mice despite High Fat Diet. ISRN Obes 2014: 540582.

Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 22: 159–168.

Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, Thomas G (2004) Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature 431: 200–205.

Wang Y, Xin X, Jin Z, Hu Y, Li X, Wu J, Jin M (2011) Anti-diabetic effects of pentamethylquercetin in neonatally streptozotocin-induced diabetic rats. Eur J Pharmacol 668: 347–353.

Wu SY, Wang GF, Liu ZQ, Rao JJ, Lü L, Xu W, Wu SG, Zhang JJ (2009) Effect of geniposide, a hypoglycemic glucoside, on hepatic regulating enzymes in diabetic mice induced by a high-fat diet and streptozotocin. Acta Pharmacol Sin 30: 202–208.

Yang Q, Guan KL (2007) Expanding mTOR signaling. Cell Res17: 666–681.

Ye L, Varamini B, Lamming DW, Sabatini DM, Baur JA (2012) Rapamycin has a biphasic effect on insulin sensitivity in C2C12 myotubes due to sequential disruption of mTORC1 and mTORC2. Front Genet 3: 177.