Original Paper

Zurampic Protects Pancreatic β -Cells from High Uric Acid Induced-Damage by Inhibiting URAT1 and Inactivating the ROS/AMPK/ERK Pathways

Ying Xin^a Kun Wang^b Zhaotong Jia^a Tao Xu^c Qiang Xu^d Jian Zhang^e
Jia Liu^d Rui Chen^d Zhongcai Du^f Jianjing Sun^b

^aDepartment of Endocrine and Metabolic Diseases, The Affiliated Hospital of Qingdao University, Qingdao, ^bDepartment of Endocrinology, Jining No.1 People's Hospital, Jining, Shandong, ^cDepartment of Respiratory Medicine, The Affiliated Hospital of Qingdao University, Qingdao, ^dDepartment of Geriatrics, The Affiliated Hospital of Qingdao University, Qingdao, ^eDepartment of Cardiology, The Affiliated Cardiovascular Hospital of Qingdao University, Qingdao, ^fDepartment of Infectious Diseases, The Affiliated Hospital of Qingdao University, Qingdao, China

Key Words

Zurampic • Urate transporter 1 • Uric acid • ROS • AMPK/ERK pathway

Abstract

Background/Aims: Zurampic is a FDA approved drug for treatment of gout. However, the influence of Zurampic on pancreatic β -cells remains unclear. The study aimed to evaluate the effects of Zurampic on high uric acid-induced damage of pancreatic β -cells and the possible underlying mechanism. **Methods:** INS-1 cells and primary rat islets were stimulated with Zurampic. mRNA expression of urate transporter 1 (URAT1) was assessed by qRT-PCR. Cells were stimulated with uric acid or uric acid plus Zurampic, and cell viability, apoptosis and ROS release were measured by MTT and flow cytometry assays. Western blot analysis was performed to evaluate the expressions of active Caspase-3 and phosphorylation of AMPK and ERK. Finally, cells were stimulated with uric acid or uric acid plus Zurampic at high levels of glucose (2.8/16.7 mM glucose), and the insulin release was assessed by ELISA. **Results:** mRNA expression of URAT1 was decreased by Zurampic in a dose-dependent manner. Uric acid decreased cell viability, promoted cell apoptosis and induced ROS release. Uric acid-induced alterations could be reversed by Zurampic. Activation of Caspase-3 and phosphorylation of AMPK and ERK were enhanced by uric acid, and the enhancements were reversed by Zurampic. Decreased phosphorylation of AMPK and ERK, induced by Zurampic, was further reduced by adding inhibitor of AMPK or ERK. Besides, uric acid inhibited high glucose-induced insulin secretion and the inhibition was rescued by Zurampic. **Conclusions:** Zurampic has a protective effect on pancreatic β -cells against uric acid induced-damage by inhibiting URAT1 and inactivating the ROS/AMPK/ERK pathway.

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Y. Xin and K. Wang contributed equally to this work.

Jianjing Sun

Department of Endocrinology, Jining No.1 People's Hospital, No.6, Jiankang Road,
Jining 272011, Shandong, (China)
E-Mail sjjstarksky@163.com

Introduction

Hydroxylation of urate, catalyzed by urate oxidase (UOX), is the first and rate-limiting step of uricolytic, which changes uric acid to more soluble metabolic allantoin [1]. Lack of UOX in liver cells makes uric acid become the main end metabolic product of purine as well as the result of self-metabolism and food. The highest level of uric acid in women is 6 mg/dL and the highest level in men is 7 mg/dL [2, 3]. Recently, high concentration of uric acid in serum, also termed hyperuricaemia, becomes a common disease requiring stringent change in life-style and diet. It also becomes the “fourth high” following with hypertension, hyperlipidemia and hyperglycemia. Long term of high uric acid can lead to multiple organ involvement and tissue loss, such as arthritis, gout, kidney disease and cardiovascular disease. In addition, high uric acid content can also damage beta islet cells, reduce the sensitivity to insulin, resulting in the high incidence of diabetes mellitus type 2 [4-9]. As of now, the treatment of hyperuricaemia mainly focuses on drugs, such as probenecid, sulfapyrazone, benzbromarone, febuxostat and xanthine oxidase inhibitor (XOI). Novel and more effective drugs are urgently needed to be investigated.

Zurampic, also termed Lesinurad, is usually recommended by physicians for the treatment of hyperuricemia combined with XOI [10]. In 2014, Zurampic is approved by US FDA for patients with gout because of the selective uric acid reabsorption inhibition [11]. Zurampic is reported to function through inhibition of the transporter 1 (URAT1) which mainly responsible for reabsorption of uric acid from the peritubular ultrafiltrate into the epithelial cells of the renal proximal convoluted tubule [12, 13]. Therefore, we mainly focused on Zurampic to explore the potential therapeutic effects and possible underlying mechanisms.

Hyperuricaemia strongly correlates with insulin resistance and abnormal glucose metabolism [9]. Pancreatic β -cells participate in glucose metabolism and exert critical core roles. Oxidative stress injury has been reported to be involved in pancreatic β -cell injury induced by uric acid. It has been demonstrated that uric acid can inhibit cell proliferation and increase oxidative stress, thereby inhibit insulin secretion by activating the reactive oxygen species (ROS)/nicotinamide monophosphate-activated protein kinase (AMPK)/extracellular signal-regulated kinase (ERK) pathways [14-18]. Likewise, a previous study performed in pancreatic β -cells has demonstrated uric acid triggers inhibition of oxidative stress and growth through activations of the AMPK and ERK signal pathways [17]. Thus, we also explored whether Zurampic could protect pancreatic β -cells against high uric acid induced-damage through modulation of the ROS/AMPK/ERK pathway.

In the present study, we attempted to evaluate the effects of Zurampic on high uric acid induced-damage in pancreatic β -cell line INS-1 and primary rat islets, along with the possible underlying mechanisms.

Materials and Methods

Cell culture and treatment

Rat INS-1 cells (Biohermes Ltd., Wuxi, China) were maintained in RPMI 1640 medium (Gibco-BR, Paisley, UK) supplemented with 10 mM glucose, 10% fetal bovine serum (FBS, Gibco), 1 mM sodium pyruvate, 10 mM HEPES, 2 mM glutamine, 50 μ M β -mercaptoethanol, 100 U/mL penicillin, and 100 μ g/mL streptomycin (all from Sigma, St. Louis, MO, USA). Primary rat islets were isolated as described previously with some modifications [19]. The procedure was approved by the ethical committee of the Affiliated Hospital of Qingdao University. In brief, Sprague-Dawley rats (male, weighing 250-300 g), purchased from Shanghai Laboratory Animal Center (Shanghai, China), were sacrificed with decapitation and the pancreas was isolated carefully. Then, a pancreas was digested by 5 mL collagenase XI (1000 U/mL; Sigma) in Hanks' balanced salt solution (HBSS) at 37°C for 16 min. After termination of digestion and islet separation by centrifugation, islets were handpicked under an inverted microscope. Primary rat islets were maintained in RPMI 1640 supplemented with 10 mM glucose, 10% fetal calf serum (Gibco-BR), 100 U/mL penicillin and

100 µg/mL streptomycin. Cells were maintained in a humidified incubator at 37°C with 5% CO₂ and 95% air. Experiments were typically performed with cells at 80% confluence. Zurampic (AstraZeneca, Wilmington, DE, USA) simulation was performed 30 min prior to addition of uric acid (15 mg/dL, Sigma). Uric acid stock solution was prepared at 15 mg/mL in 0.5 M NaOH. For inhibition of AMPK or ERK, cells were pretreated with AMPK inhibitor (compound C, 10 µM) or ERK inhibitor (PD98059, 20 µM) for 30 min prior to addition of uric acid.

Quantitative reverse transcription PCR (qRT-PCR) of URAT1

After incubation with various concentrations of Zurampic for 24 h, total RNA was isolated from H4IIE cells and primary rat islets by using TRIzol reagent, isopropanol and ethanol according to the manufacturer's protocol (all from Sigma). Then, cDNA was synthesized after reverse transcription with MultiScribe RT (Applied Biosystems, Foster City, CA, USA) in line with supplier's instructions. Real-time PCR was performed with SYBR Green PCR Master Mix (Applied Biosystems) following the instructions of manufacturer. Primers for URAT1 are: 5'-GAG GCA GAC ACG TTG ACT GT-3' (forward) and 5'-TTG CTT CTT TCT TCT TGC A-3' (reverse), and both were synthesized by Sangon (Shanghai, China). The URAT1 amplification conditions were as follows: pre-denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 15 s, annealing and extension at 61°C for 30 s, and then melting curve analysis at temperatures of 60-95°C. Each sample was assessed 3 times [20]. Relative expression was calculated on the basis of the $2^{-\Delta\Delta C_T}$ method [21], normalizing to GAPDH expression.

MTT assay for cell viability

Cell viability of INS-1 cells and primary rat islets treated with uric acid or uric acid plus Zurampic were determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded with a density of 2×10^4 into 96-well plates and treated with 15 mg/dL uric acid or uric acid plus Zurampic. At 0, 24, 48, 72 and 96 h after treatment, culture medium was discarded and 100 μ L MTT solution (0.5 mg/mL, Sigma) was added to each well, followed by incubation at 37°C for 4 h. Then, MTT solution was removed and 150 μ L DMSO was added to dissolve formazan crystals. The absorbance was measured at 492 nm on a microplate reader.

Cell apoptosis detection

Cell apoptosis was evaluated by Annexin V-FITC/propidium iodide (PI) apoptosis detection kit (BD Biosciences, San Diego, CA, USA). INS-1 cells and primary rat islets were plated in 6-well plates with a density of 1×10^6 cells per well and incubated under appropriate conditions (uric acid or uric acid plus Zurampic). The incubation and treatment of uric acid was lasted for 24 h. To make single cell staining, cells were treated with EDTA and centrifuged at $150 \times g$ for 3 min at 4°C . After aspirating supernatants, cells were washed with ice-cold Annexin V binding solution and centrifuged at $200 \times g$ for 3 min at 4°C . Supernatants were removed and 3 μL of Annexin V-FITC and 10 μL of PI were added. After incubation for 15 min in the dark, 1 mL of binding buffer was added and the mixture was detected by an LSR II flow cytometer (BD Biosciences). The results were analyzed by FlowJo software (Tree Star, San Carlos, CA, USA).

Assessment by flow cytometry

cells and primary rat islets were seeded into 6-well plates (2.5×10^5 cells/well) and maintained at 37°C for 24 h. Then, cells were exposed to uric acid (15 mg/dL) and uric acid plus Zurampic for 24 h, and stained with $10 \mu\text{M}$ 2, 7-dichlorodihydrofluorescein-diacetate (DCFDA, Sigma) for 30 min at 37°C as previously described [17]. Stained cells were analyzed by an LSR fluorescent assisted cell sorting (FACS) machine (BD Biosciences) at emission wave-length 480 nm; excitation wave-length 530 nm. Data were given as percentage of cells in region "M". Each treatment condition contained three independent experiments.

Western blot analysis

After incubation with uric acid for 30 min, cells were scraped off with lysis buffer composed of 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na₃VO₄, 1 μg/mL leupeptin, 1 mM phenylmethanesulfonyl fluoride (PMSF) and phosphatase inhibitors (phosphatase inhibitor mixture I). The supernatant protein concentration was determined by BCA Protein Assay Kit (Pierce, Rockford, IL, USA), and equivalent amount of proteins (80

μg) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After that, proteins were transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA), followed by blocking with 5% non-fat milk and incubation with primary antibodies against phospho-ERK (p-ERK), ERK (both from Bioworld, St. Louis Park, MN, USA), phospho-AMPK (p-AMPK), AMPK, Caspase-3 and GAPDH (all from Cell Signaling Technology, Beverly, MA, USA). After rinsing, membranes were incubated with horseradish peroxidase-conjugated secondary antibody. Finally, an enhanced chemiluminescence kit (Pierce) was used for signal detection.

Enzyme-linked immunosorbent assay (ELISA) for glucose-stimulated insulin secretion

INS-1 cells and primary rat islets with a density of 2×10^5 cells/well were seeded into 96-well plates and cultured at 37°C for 48 h. Then, cells were washed with PBS and preincubated in 1 mL KRBBH bicarbonate HEPES buffer (KRBB: 115 mM NaCl, 24 mM NaHCO₃, 5 mM KCl, 1 mM MgCl₂, 15 mM HEPES, 0.5% BSA, pH 7.4) containing 2.8 mM glucose for 30 min with uric acid (15 mg/100 μL) or uric acid plus Zurampic. For glucose stimulation, the buffers were replaced by 1 mL KRBBH with uric acid (15 mg/100 μL) or uric acid plus Zurampic containing 16.7 mM glucose. After an additional incubation for 60 min, insulin concentration in supernatants of cell culture was determined by a rat insulin ELISA kit (Mercodia, Uppsala, Sweden) according to the protocol suggested by the supplier.

Statistical analysis

All the data were expressed as means ± SD of at least three independent experiments. Statistical analysis was performed using Graphpad Prism 5 software (GraphPad, San Diego, USA). The *P*-values were analyzed by two-tailed unpaired *t* test or two-way analysis of variance (ANOVA) with Bonferroni's correction. Differences of *P* < 0.05 were considered statistically significant.

Results

URAT1 expression is decreased by Zurampic stimulation in a dose-dependent manner

After being stimulated by different concentrations of Zurampic (0, 5, 10, 25 and 50 μg/mL), URAT1 expression was assessed by qRT-PCR. In Fig. 1A, the expression of URAT1 was significantly down-regulated by 5–50 μg/mL Zurampic (*P* < 0.05) in INS-1 cells. When compared to stimulation with 25 μg/mL Zurampic, the decrease of URAT1 expression was not statistically significant in the 50 μg/mL Zurampic group (*P* > 0.05). Meanwhile, in Fig. 1B, expression of URAT1 was significantly down-regulated by 10–50 μg/mL Zurampic (*P* < 0.05) in primary rat islets. As compared to the 0 μg/mL Zurampic group, the decrease of URAT1 expression was not significant in the 5 μg/mL Zurampic group (*P* > 0.05). We concluded that URAT1 expression was decreased by Zurampic stimulation in a dose-dependent manner, and the concentration of Zurampic for further experiments was 25 μg/mL.

Uric acid-induced cell viability by uric acid is abrogated by Zurampic

After treatments with uric acid or uric acid plus Zurampic for 96 h, cell viability was assessed by MTT assay. In Fig. 2A–2B, cell viability of INS-1 cells and primary rat islets was significantly decreased by uric acid stimulation compared with non-treated cells at 24 h (both *P* < 0.05), 48 h (*P* < 0.01 or *P* < 0.001), 72 h (*P* < 0.01 or *P* < 0.001), and 96 h (both

Fig. 1. URAT1 expression is decreased by Zurampic in a dose-dependent manner. INS-1 cells (A) and primary rat islets (B) were stimulated by Zurampic at 0, 5, 10, 25, or 50 μg/mL, and then URAT1 mRNA expression was evaluated by qRT-PCR. Data are presented as mean ± SD of three independent experiments. ns, *P* > 0.05; *, *P* < 0.05; **, *P* < 0.01. URAT1, urate transporter 1.

Fig. 2. Zurampic reverses the uric acid-induced inhibition of cell viability. INS-1 cells (A) and primary rat islets (B) were stimulated with vehicle, uric acid or uric acid plus Zurampic, and then cell viability was assessed by MTT assay. Data are presented as mean $\pm$ SD of three independent experiments. * referred to the significance of comparison between uric acid-treated and non-treated cells. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. # referred to the significance of comparison between uric acid-treated and uric acid plus Zurampic-treated cells. #, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$. MTT, 3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide (MTT).

$P < 0.001$) post-stimulation. However, Zurampic stimulation markedly increased cell viability compared with cells treated by uric acid alone at 24 h ($P < 0.05$ or $P < 0.01$), 48 h ($P < 0.01$ or $P < 0.001$), 72 h ($P < 0.01$ or $P < 0.001$), and 96 h (both $P < 0.001$) post-stimulation. The results suggested that uric acid reduced cell viability and the reduction could be reversed by Zurampic.

Enhanced cell apoptosis by uric acid is abrogated by Zurampic

After treatment with uric acid or uric acid plus Zurampic, cell apoptosis was assessed by flow cytometry. Results in Fig. 3A-3D show that uric acid significantly increased apoptosis of INS-1 cells and primary rat islets as compared with non-treated cells ($P < 0.01$ or $P < 0.001$), whereas Zurampic abrogated the increases of cell apoptosis induced by uric acid when compared to cells treated by uric acid alone (both $P < 0.01$). Moreover, the Western blot analysis showed that the expression of cleaved Caspase-3 was obviously up-regulated by uric acid while the up-regulation was reversed by Zurampic in both INS-1 cells (Fig. 3E) and primary rat islets (Fig. 3F). Hence, we drew a conclusion that uric acid promoted cell apoptosis while the promotion could be abrogated by Zurampic.

ROS increase induced by uric acid is reduced by Zurampic

After treatments with uric acid or uric acid plus Zurampic, ROS level was assessed by FACS. In INS-1 cells (Fig. 4A-4B) and primary rat islets (Fig. 4C-4D), ROS level was significantly

Fig. 3. Zurampic protects INS-1 cells and primary rat islets against uric acid-induced apoptosis. Cells were stimulated with vehicle, uric acid or uric acid plus Zurampic. A-B, Apoptosis of INS-1 cells by flow cytometry. C-D, Apoptosis of primary rat islets by flow cytometry. E, Protein expression of cleaved Caspase 3 in INS-1 cells by Western blot analysis. F, Protein expression of cleaved Caspase 3 in primary rat islets by Western blot analysis. Data are presented as mean $\pm$ SD of three independent experiments. **, $P < 0.01$; ***, $P < 0.001$.

enhanced by uric acid treatments compared with non-treated cells (both $P < 0.01$), whereas the increases of ROS level were abrogated by Zurampic compared with cells treated with uric acid alone (both $P < 0.01$). Results illustrated that uric acid increased the ROS level while the increases were reversed by simulation of Zurampic.

Activations of AMPK and ERK induced by uric acid are reversed by Zurampic

After treatments with uric acid, Zurampic and the inhibitors of AMPK (compound C) and ERK (PD98059), expression and phosphorylation of AMPK and ERK were assessed by Western blot analysis. In Fig. 5A-5B, phosphorylation levels of AMPK and ERK were both significantly up-regulated by uric acid (both $P < 0.01$) while these up-regulations were markedly abrogated by Zurampic (both $P < 0.01$) in both INS-1 and primary rat islets. Compared to the uric acid group, phosphorylation of AMPK and ERK was dramatically down-regulated by compound C ($P < 0.01$ or $P < 0.001$) and PD98059 ($P < 0.01$ or $P < 0.001$), respectively, in both INS-1 cells and primary rat islets. Moreover, the down-regulated AMPK and ERK, induced by Zurampic, were reversed by compound C or PD98059 as compared to the uric acid + Zurampic group (both $P < 0.001$). Therefore, we concluded that Zurampic might function through inhibition of AMPK and ERK.

Reduction of insulin secretion induced by uric acid is rescued by Zurampic

After treatments with uric acid or uric acid plus Zurampic, along with 2.8 mM glucose and 16.7 mM glucose, the insulin secretion was evaluated by ELISA. ELISA results showed that insulin concentration of cells treated with 16.7 mM glucose was significantly higher than that treated with 2.8 mM glucose in both INS-1 cells ($P < 0.01$ or $P < 0.001$, Fig. 6A) and primary rat islets ($P < 0.05$ or $P < 0.001$, Fig. 6B). Under treatments with 16.7 mM glucose, insulin concentration was markedly reduced by uric acid compared with non-treated cells ($P < 0.05$), while the reduction was rescued by Zurampic compared with cells treated with uric acid ($P < 0.05$), in both INS-1 cells and primary rat islets. The results suggested that Zurampic had a protective effect on insulin secretion.

Discussion

Zurampic, an inhibitor of URAT1, is a US FDA approved drug which recommended for the treatment of gout when combined with XO1. However, survey revealed the influence

Fig. 4. Zurampic suppresses reactive oxygen species (ROS) release induced by uric acid in INS-1 cells (A-B) and primary rat islets (C-D) were stimulated with uric acid, uric acid or uric acid plus Zurampic, and ROS level was evaluated by flow cytometry assisted cell sorting (FACS). Data are presented as mean $\pm$ SD from three independent experiments. ** $P < 0.01$. DCFA, 2,2'-dichlorodihydrofluorescein-diacetate.

Fig. 5. Zurampic inhibits uric acid-induced activations of AMPK and ERK. **A.** INS-1 cells and primary rat islets were stimulated with vehicle, uric acid, uric acid plus Zurampic, uric acid plus compound C or uric acid plus Zurampic plus compound C, and phosphorylation levels of AMPK were assessed by Western blot analysis. **B.** INS-1 cells and primary rat islets were stimulated with vehicle, uric acid, uric acid plus Zurampic, uric acid plus PD98059 or uric acid plus Zurampic plus PD98059, and phosphorylation levels of ERK were assessed by Western blot analysis. The relative phosphorylation was expressed as the relative intensity of phosphorylated kinases/total kinases and the results were normalized by GAPDH. In addition, the final results of each group were also normalized to the data of the NC group. Data are presented as mean $\pm$ SD of three independent experiments. ** $P < 0.01$; *** $P < 0.001$. AMPK: adenosine monophosphate-activated protein kinase; ERK, extracellular signal-regulated kinase; p-, phosphorylated.

Fig. 6. Zurampic rescues abnormal insulin secretion induced by uric acid. INS-1 cells (A) and primary rat islets (B) were stimulated with vehicle, uric acid or uric acid plus Zurampic at low glucose (2.8 mM) and high glucose (16.7 mM) conditions, and the insulin concentration was measured by enzyme-linked immunosorbent assay (ELISA). Data are presented as mean $\pm$ SD of three independent experiments. * indicates the significance of comparison between low

glucose-treated and high glucose-treated cells. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. # indicated the significant difference compared with uric acid-treated cells at high glucose level; #, $P < 0.05$.

Zurampic on pancreatic β -cells is yet to be established. In our study, we validated that Zurampic could down-regulate URAT1 mRNA expression in INS-1 cells and primary rat islets, and the most appreciate concentration was 25 μ M. The followed experiments demonstrated uric acid decreased cell viability and promoted cell apoptosis. In addition, uric acid significantly increased ROS level, along with activations of AMPK and ERK. Furthermore, 16.7 mM glucose-induced insulin secretion could be repressed by uric acid. Above all, we interestingly identified that all the effects of uric acid on INS-1 cells and primary rat islets could be abrogated by Zurampic.

Accumulating literature has proposed that uric acid is involved in cell viability and apoptosis. A previous study once implied that uric acid decreased proximal tubular cell viability [22]. Another study gave the opposite evidence which proved suitable concentration of uric acid improved cell viability of HT22 hippocampal cells and BV-2 microglia [23]. Thus,

the effects of uric acid on cell viability are varied depending on cell types. In our study, the cell viability of INS-1 cells and primary rat islets was significantly inhibited by uric acid. As for cell apoptosis, uric acid is reported to act as a promoter in human proximal tubule cells [22]. Meantime, hyperuricemia is proved to cause pancreatic β -cell death and dysfunction [24]. The results in our study were consistent with previous studies, indicating that uric acid obviously promoted cell apoptosis in INS-1 cells and primary rat islets. Considering the approved application of Zurampic in hyperuricemia, we explored the effects of Zurampic on uric acid-induced alterations of cell viability and apoptosis in INS-1 cells and primary rat islets. The results interestingly indicated Zurampic could reverse the effect of uric acid on cell viability and apoptosis.

Uric acid has been reported to promote apoptosis by oxidative stress in human proximal tubule cells [22]. Yu et al. showed that uric acid inhibited cell proliferation and induced senescence and apoptosis by local activation of oxidative stress and angiogenesis in system in endothelial cells [25]. Therefore, we further explored the ROS level in INS-1 cells and primary rat islets treated with uric acid or uric acid plus Zurampic. Our study showed the ROS level was markedly increased by uric acid which consisted with results reported previously. Moreover, Zurampic stimulation was proved to effectively decrease the increase of ROS level induced by uric acid.

ERK is one of the downstream targets for oxidative stress. According to previous studies showed that uric acid activated ERK phosphorylation in vascular smooth muscle cells and adipocytes [26, 27]. Simultaneously, AMPK is another target of oxidative stress. An array of reports demonstrated that AMPK was activated by oxidative stress in vascular endothelial and skin epidermal cells [28–30]. Observations in our study were consistent with these studies, showing that phosphorylation levels of AMPK and ERK were changed with the alterations of ROS level. Zhang *et al.* once reported uric acid induced oxidative stress and growth inhibition by activations of the AMPK and ERK signaling pathway [17]. In our study, the addition of the inhibitors of AMPK and ERK reinforced that Zurampic protected INS-1 cells and primary rat islets from uric acid-induced damage through inactivating AMPK and ERK.

Moreover, hyperuricaemia is closely involved in insulin resistance and abnormal glucose metabolism [9]. In terms of pancreatic β -cells are the producers of insulin, thus the abnormal insulin secretion under the stimulation might be a common damage induced by uric acid. The results in our study were consistent with results by Rocic *et al.*, showing that uric acid inhibited glucose-stimulated insulin secretion in pancreatic β -cells [31]. No surprisingly, Zurampic reversed the abnormal insulin secretion caused by uric acid in INS-1 cells and primary rat islets. Moreover, we found in our study there was no significant effect of uric acid on basal insulin release. However, the basal insulin release in the literature by Rocic *et al.* was inhibited by uric acid, which might contribute to the difference of glucose concentration in the medium. The basal medium used in our study contained 2.8 mM glucose, whereas that in the previous literature contained 3.3 mM glucose. The slight difference of glucose concentration might be the reason for explaining the different results in our study and the previous study.

Together, we interestingly identified that Zurampic could protect INS-1 cells and primary rat islets against uric acid-induced damage on cell viability and apoptosis, ROS release, and insulin secretion through inhibiting URAT1 expression and inactivating the AMPK/ERK signaling pathways. The results might provide novel therapeutic strategies for high uric acid-induced diseases.

Disclosure Statement

The authors declare that they have no conflict of interests.

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