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β-Hydroxybutyrate, a ketone body, reduces the cytotoxic effect of cisplatin via activation of HDAC5 in human renal cortical epithelial cells

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ABSTRACT

Aims: β-Hydroxybutyrate (βOHB) is a metabolic intermediate that constitutes about 70% of ketone bodies produced in liver from oxidation of fatty acids released from adipose tissue. A recent study showed that βOHB inhibits HDAC1, 3 and 4 (classes I and IIa) in human embryonic kidney 293 (HEK293) cells. Therefore, βOHB could regulate epigenetics via modulating HDACs. However, little is known about the protective effect of βOHB on renal cells through epigenetics. The aim of this study is to investigate whether βOHB reduces cisplatin-induced nephrotoxicity in human renal cortical epithelial (HRCE) cells by modulating HDACs.

Main methods: In this study, we used human renal cortical epithelial (HRCE) cells. The anti-apoptotic effect of βOHB was evaluated using flow cytometry analysis. The expression of apoptosis-related proteins and HDACs was evaluated by western immunoblot.

Key findings: The results showed that βOHB significantly reduced cisplatin-induced apoptosis in HRCE cells. Furthermore, βOHB significantly reduced cisplatin-induced cleavage of caspase-3, acetylation of histone H3, and phosphorylation of AMP-activated kinase. This anti-apoptotic effect of βOHB was markedly attenuated by an inhibitor of HDAC4/5, and βOHB-mediated suppression of cleavage of caspase3 was significantly blocked by siRNA-induced gene silencing of HDAC5.

Significance: βOHB attenuates cisplatin-induced apoptosis by activation of HDAC5 in HRCE cells, suggesting that βOHB may be a new therapeutic agent for cisplatin nephropathy.

1. Introduction

Ketone bodies such as β-hydroxybutyrate (βOHB) are fatty acid-derived molecules that are mainly produced in the liver and serve as a circulating energy source for peripheral tissues during fasting or exercise. In humans, the basal serum level of βOHB is in the low micromolar range, but this level can reach 1–2 mM after 2 days of fasting [1,2] and 6–8 mM after prolonged starvation [3]. A level of βOHB of 1–2 mM can also be reached after intense exercise for 90 min [4].

βOHB has recently been shown to inhibit endogenous histone deacetylases (HDACs): HDAC 1, 3, and 4 (classes I and IIa) [5]. HDACs are a family of proteins that regulate gene expression, including genes involved in glucose metabolism [6]. Histone hyperacetylation relaxes chromatin and is associated with activation of gene transcription; conversely, histone deacetylation suppresses gene transcription. HDACs are major players in accelerating histone deacetylation; therefore, βOHB can regulate chromatin structure and epigenetics via HDACs.

Neuroprotective effects of βOHB have also been reported. It has been known for many years that children with drug-resistant refractory epilepsy improve on a strict low carbohydrate ketogenic diet (LCKD), and recent data showed that βOHB supplementation protects neurons in models of Alzheimer's and Parkinson's disease [7–9]. Cardioprotective effects of βOHB have also been observed using in vivo ischemia/reperfusion approaches in rats subjected to starvation-induced ketosis and via intravenous injection of βOHB prior to ischemic injury, which conferred a significant decrease in both infarct size and myocardial cell death [10,11]. Moreover, it has been shown that rats on a LCKD have a remarkable tolerance to ischemia and a faster recovery of cardiac

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function following reperfusion [12]. In contrast, little is known about the protective effect of βOHB on renal cells.

Cisplatin, which inhibits DNA replication by forming intra- and interstrand crosslinks between purine bases, is a highly effective antitumor drug used in standard chemotherapies for solid malignant tumors, including head and neck, esophageal, lung, ovarian, and testicular cancers [13]. However, clinical use of cisplatin is limited because of an adverse effect of nephrotoxicity, with about 10% of patients developing acute kidney injury (AKI) after an initial dose of cisplatin [14]. This is also of concern because AKI may increase the risk for chronic kidney disease (CKD) [15,16]. Therefore, finding a novel therapeutic agent to minimize cisplatin-induced nephropathy is required to improve cancer chemotherapy. In this study, we investigated whether βOHB inhibits cisplatin-induced nephrotoxicity in a HDAC-dependent manner in human renal cortical epithelial (HRCE) cells.

2. Materials and methods

2.1. Material

DL-3-Hydroxybutyric acid sodium salt (Sigma-Aldrich, St. Louis, MO, USA); triciribine (Wako, Osaka, Japan); trichostatin A (TSA) (Tokyo Chemical Industry, Tokyo, Japan); LMK-235, Compound C (Selleckchem, Houston, TX, USA); polyclonal rabbit antibodies against human β-actin, Bcl-2, RIP3, phospho-HDAC5 (Abcam), acetyl-histone H3 (Lys9/Lys14), cleaved caspase-3 (Asp175), cleaved caspase-9, p53, AMPK (all Cell Signaling Technology, Boston, MA, USA); monoclonal rabbit antibodies against HDAC1, HDAC2, HDAC4, HDAC5, HDAC6, HDAC7, phospho-AMPK (Thr172) (Cell Signaling Technology) and HDAC8 (Abcam); monoclonal mouse antibodies against HDAC3, caspase-8 (Cell Signaling Technology) and phospho-RIP3 (T231/S232) (Abcam); and horseradish peroxidase-conjugated anti-mouse, anti-goat and anti-rabbit immunoglobulins (Dako, Glostrup, Denmark) were used in the study.

2.2. Cell culture

HRCE cells (Lonza Walkersville, Inc., Walkersville, MD, USA) were grown in renal cell growth medium (REGM). When cells reached a logarithmic phase of growth, they were digested with trypsin and seeded in culture plates as required. Confluent cells (passages 4–6) were incubated for 24 h in Dulbecco's modified Eagle's medium (DMEM) supplemented with 0.5% FBS before stimulation experiments at 37 °C in a humidified atmosphere containing 5% CO_2 and 18% O_2. When HRCE cells reached confluent, HRCEs cells were stimulated with various concentrations of chemical materials for 24 to 48 h. The concentration and time of incubation of HRCE cells with chemical materials was described in each Figure/Figure legends. The final concentration of DMSO in the medium was < 0.1%. Vehicle (0.1% DMSO) was added to the control samples.

2.3. Knockdown of HDAC5 in HRCE cells

Gene silencing of HDAC5 in HRCE cells was performed as described previously [17]. Small interfering RNA (siRNA) against HDAC5 (ON-TARGET plus Human HDAC5), control siRNA (ON-TARGET plus Non-targeting) were purchased from GE Healthcare Dharmacon (Lafayette, CO, USA). HRCE cells (70% confluence) were transfected with control siRNA or siRNA against HDAC5 at a final concentration of 10 nmol/L, using a transfection reagent (DharmaFECT; Dharmacon). After 48-h incubation, the cells were refreshed with DMEM and then treated for a further 48 h with cisplatin with or without βOHB.

2.4. Immunoblotting

HRCE cells were lysed in RIPA buffer with phosphatase inhibitors (Sigma-Aldrich). Lysates (10 μg protein) were analyzed by immunoblot analysis, first using antibodies targeting β-actin (1:1000), cleaved caspase-3 (1:1000), caspase-8 (1:1000), caspase-9 (1:1000), acetyl-H3 (1:1000), pAMPK (1:1000), AMPK (1:1000), Bax (1:1000), Bcl-2 (1:1000), RIP3 (1:1000), pRIP3 (1:1000), p53 (1:1000), Acetyl-H3 (1:1000), HDAC1 (1:1000), HDAC2 (1:1000), HDAC3 (1:1000), HDAC4 (1:1000), HDCA5 (1:1000), HDAC6 (1:1000), HDAC7 (1:1000), and HDAC8 (1:5000) for 24 h; and then with appropriate horseradish peroxidase-conjugated secondary antibodies (1:1000) at room temperature for 1 h. Immunoreactive bands were visualized as previously reported [17].

2.5. Cell apoptosis quantification

An annexin V kit (MBL, Nagoya, Japan) was used to evaluate apoptosis in HRCE cells. Cells were resuspended in 85 μl of binding buffer. The suspension was incubated with 10 μl of annexin V-FITC and 5 μl of propidium iodide (PI) for 15 min at room temperature in the dark. Then, 400 μl of binding buffer was added to each tube and the percentage of apoptotic cells was quantified by flow cytometry using a BD FACSCanto™ II Flow Cytometer.

2.6. Murine model of cisplatin-induced AKI

Animals were housed in appropriate pathogen-free conditions. All experimental procedures conformed to the Regulations for Animal Research at the University of Fukui, and were reviewed by the Animal Research Committee of the University of Fukui. Cisplatin nephrotoxic AKI was induced in male mice (age 8–10 weeks). Mice were intraperitoneally injected with a single dose of cisplatin at 20 mg/kg. All mice received a total volume of 10 μl/g normal saline or 500 mg/kg of DL-3-hydroxybutyric acid sodium salt 1 h after the injection of cisplatin. All groups in which βOHB was applied received repeated 500 mg/kg of DL-3-hydroxybutyric acid sodium dissolved in normal saline injections every 24 h, with comparison groups receiving repeated injections of 10 μl/g normal saline. Blood samples were collected at day 3 after cisplatin injection to measure serum creatinine and serum blood urea nitrogen (BUN).

2.7. Statistical analysis

Data of in vitro are expressed as the mean ± standard deviation (SD). Data of in vivo are expressed as the mean ± standard error (SE). The significance of differences between two groups was evaluated by Student *t*-test and that among more than two groups was assessed using analysis of variance (ANOVA) with a Tukey-Kramer multiple comparison test. Results were considered to be significant at $P < 0.05$.

3. Results

3.1. Effects of βOHB combined with cisplatin on murine model of cisplatin-induced AKI

To determine the contribution of ketone bodies to cisplatin-induced AKI model in mice, we investigated the effects of βOHB to ameliorate the loss of kidney function. The elevations in the levels of serum creatinine (Fig. 1A) and serum BUN (Fig. 1B) were significantly attenuated in βOHB-treated mice.

3.2. βOHB reduces cisplatin-induced apoptosis via an intrinsic pathway in HRCE cells

Cisplatin-induced AKI is associated with renal tubular cell death, which leads to renal dysfunction. Many previous studies indicates that apoptosis is the main pathological cause for cisplatin-induced renal tubular damage [18]. Apoptosis is regulated by highly coordinated

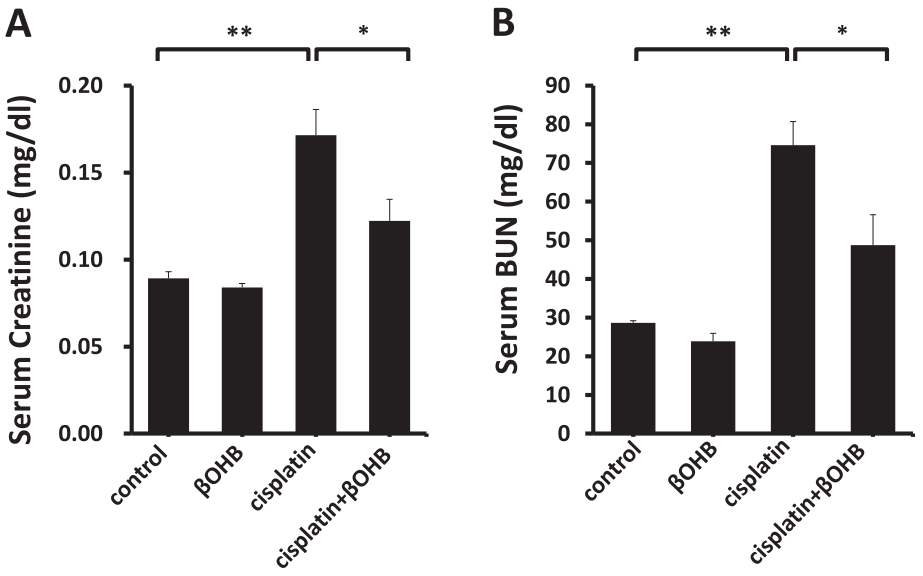

Fig. 1. Effects of βOHB combined with cisplatin on murine model of cisplatin-induced AKI. Male C57BL/6 mice underwent intraperitoneal injection with vehicle or 20 mg/kg cisplatin (n = 5). All mice received a total volume of 10 μl/g normal saline or 500 mg/kg of DL-3-hydroxybutyric acid sodium salt 1 h after the injection of cisplatin. All groups in which βOHB was applied received repeated 500 mg/kg of DL-3-hydroxybutyric acid sodium dissolved in normal saline injections every 24 h, with comparison groups receiving repeated injections of 10 μl/g normal saline. Blood samples were collected at day 3 after cisplatin injection to measure serum creatinine (A) and serum BUN (B). $*P < 0.05$, $**P < 0.01$ by one-way ANOVA with a Tukey-Kramer multiple comparison test.

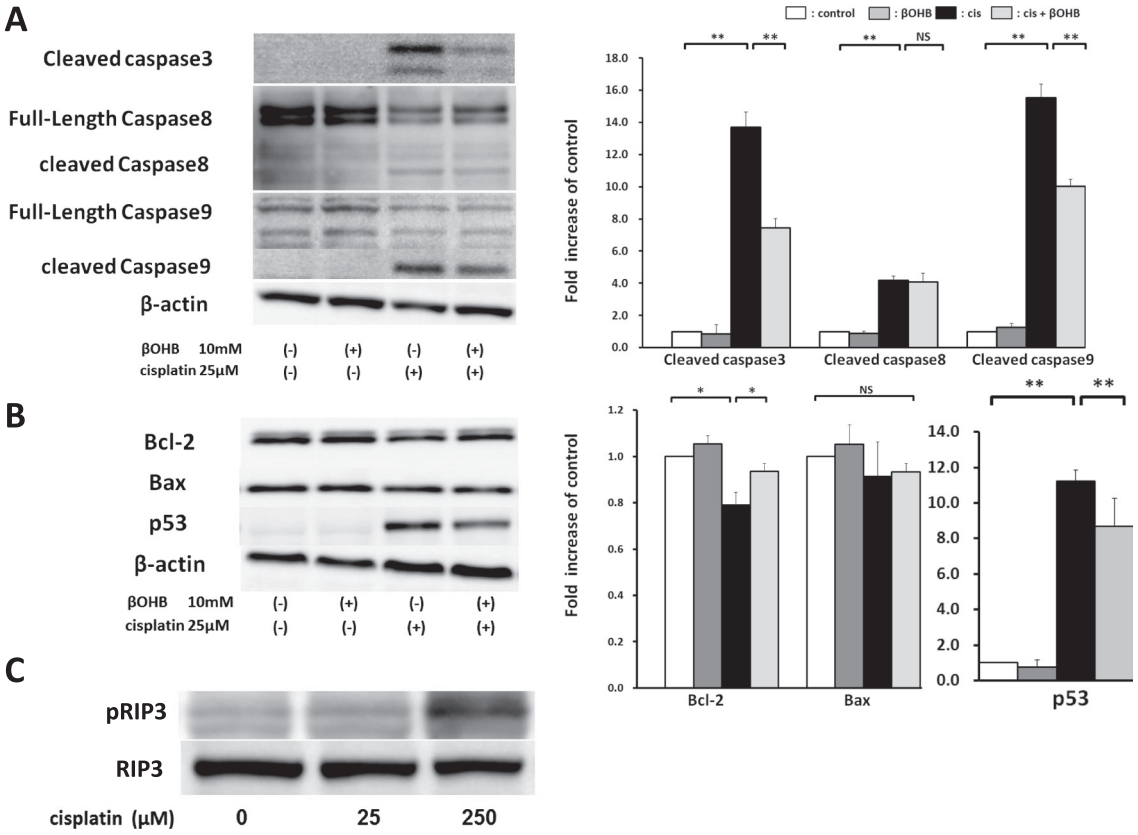

Fig. 2. Reduction of cisplatin-induced apoptosis by βOHB in HRCE cells. (A, B) HRCE cells were treated with cisplatin (25 μM) with or without βOHB (10 mM) for 48 h. Cell lysates were analyzed by western blot for apoptosis-related proteins: cleaved caspase-3, cleaved caspase-8, cleaved caspase-9, Bcl-2, Bax, and p53. The cleaved caspase-3, -8, and -9, Bcl-2, Bax, and p53 level relative to β-actin in control cells was set to 1.0. (C) HRCE cells were treated with or without cisplatin (25 μM, 250 μM) for 24 h. Cell lysates were analyzed by western blot for necroptosis-related proteins: RIP3 and phospho-RIP3. Data are expressed as the mean ± SD of three independent experiments. $*P < 0.05$, $**P < 0.01$, NS: not significant by one-way ANOVA with a Tukey-Kramer multiple comparison test.

processes that involve the activation of caspases, a family of cysteine proteases, in which caspase-3 activation is known to be responsible for DNA fragmentation and apoptosis [19,20]. We, thus, measured the cleaved, active form of caspase-3 in HRCE cells by Western blot analysis. The protein level of activated caspase-3 with βOHB + cisplatin was significantly lower than that with cisplatin alone (Fig. 2A). Next, to investigate the molecular mechanism of attenuation of cisplatin-induced apoptosis by βOHB in HRCE cells, immunoblotting was performed for caspase family members that might function during apoptosis (Fig. 2A, B). Cisplatin treatment induced cleavage of caspase-8 and -9, and the protein level of activated caspase-9 with βOHB + cisplatin was significantly lower than that with cisplatin alone (Fig. 2A). βOHB also significantly enhanced expression of Bcl-2, an anti-apoptotic protein. Furthermore, expression of p53, which reflects DNA damage, was

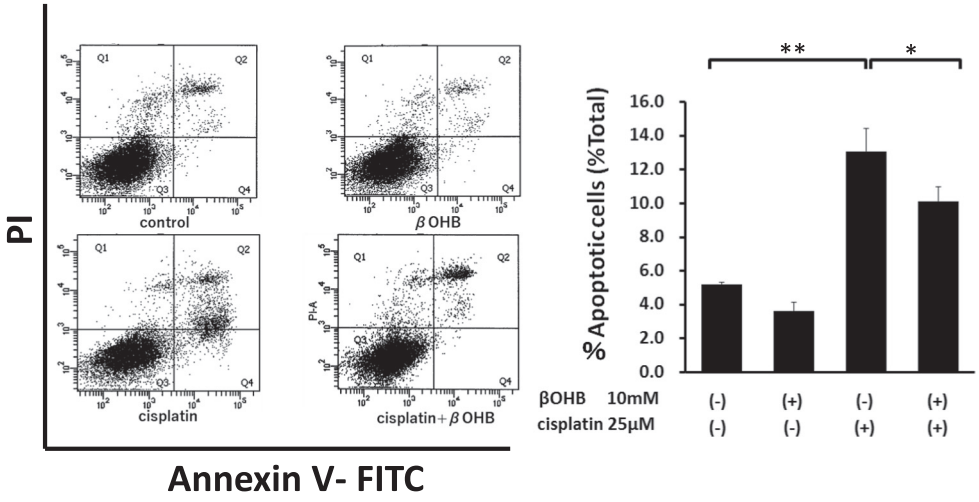

Fig. 3. Effects of βOHB combined with cisplatin on apoptosis of HRCE cells. HRCE cells were treated with cisplatin (25 μM), βOHB (10 mM), or both agents for 48 h. Cells were then stained with annexin V and PI, followed by cytometry analysis. The percentage of cells that were Annexin V-positive were represented as the portion of apoptotic cells. Data are shown as mean ± SD of % apoptosis from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ by one-way ANOVA with a Tukey-Kramer multiple comparison test.

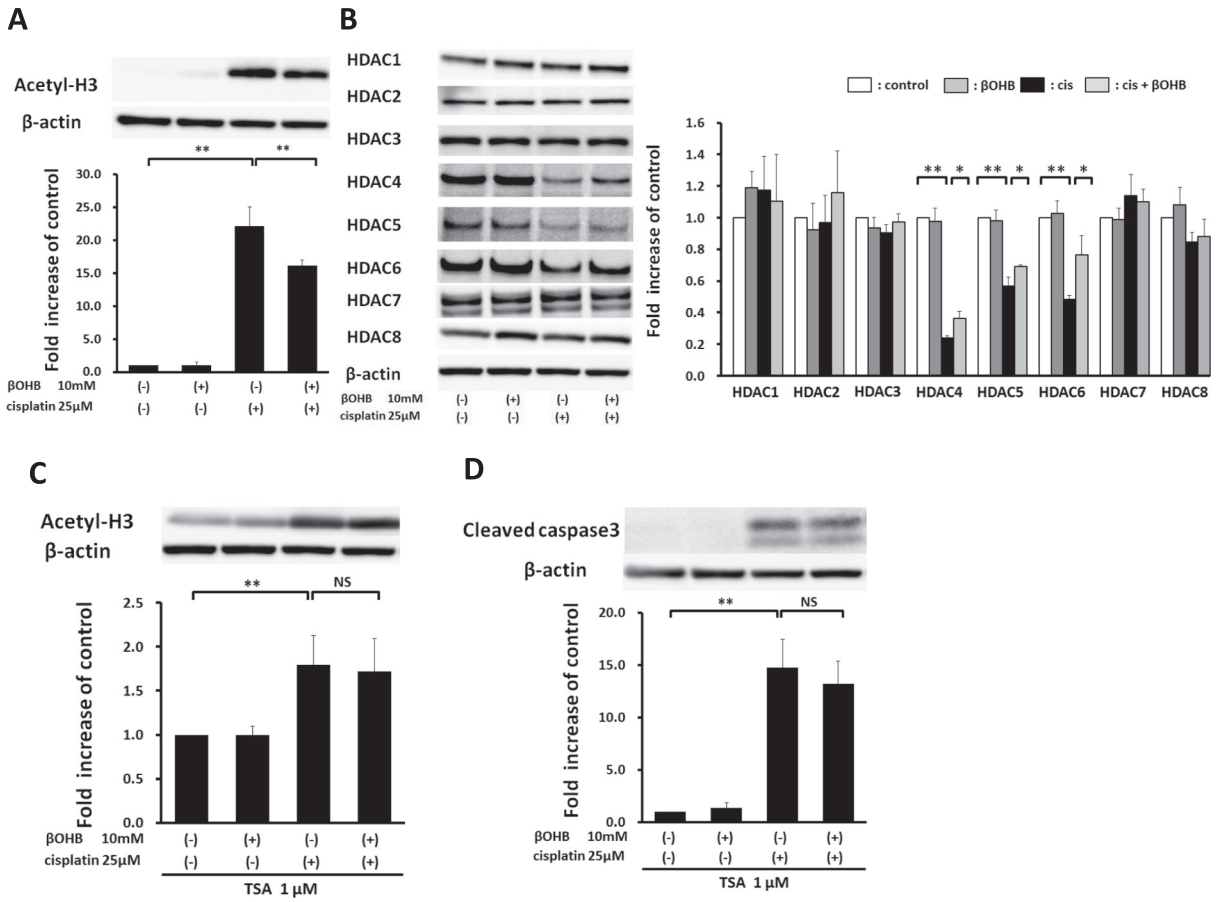

Fig. 4. βOHB reduced cisplatin-induced apoptosis by modifying HDAC in HRCE cells. (A) HRCE cells were treated with vehicle or cisplatin (25 μM) with or without βOHB (10 mM) for 48 h. Cell lysates were analyzed by western blot for acetyl histone H3. The acetyl histone H3 level relative to β-actin in control cells was set to 1.0. (B) HRCE cells were treated with cisplatin (25 μM) with or without βOHB (10 mM) for 48 h. Cell lysates were analyzed by western blot for HDAC1, 2, 3, 4, 5, 6, 7, and 8. The levels of HDACs (all relative to β-actin) in control cells were set to 1.0. (C) HRCE cells were treated with vehicle or cisplatin (25 μM) with or without βOHB (10 mM) in the presence of TSA (1 μM) for 48 h. Cell lysates were analyzed by western blot for acetyl histone H3. The acetyl histone H3 level relative to β-actin in control cells was set to 1.0. (D) HRCE cells were treated with vehicle or cisplatin (25 μM) with or without βOHB (10 mM) for 48 h. The cleaved caspase-3 level relative to β-actin in control cells was set to 1.0. Data are expressed as the mean ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, NS: not significant by one-way ANOVA with a Tukey-Kramer multiple comparison test.

significantly lower with βOHB + cisplatin than with cisplatin alone (Fig. 2B).

In addition to apoptosis, necrosis is proposed to contribute also to the cell death in renal tubules [21]. A recent study showed that necroptosis, a type of programmed necrosis, is involved in cisplatin-induced nephrotoxic AKI [22]. Therefore, we interrogated the phosphorylation of receptor-interacting protein 3 (RIP3) as the core components of the necroptotic pathway. The phosphorylation of RIP 3was not significantly induced in the treatment with cisplatin at 25 μM, however, it was significantly induced at 250 μM (Fig. 2C). These results

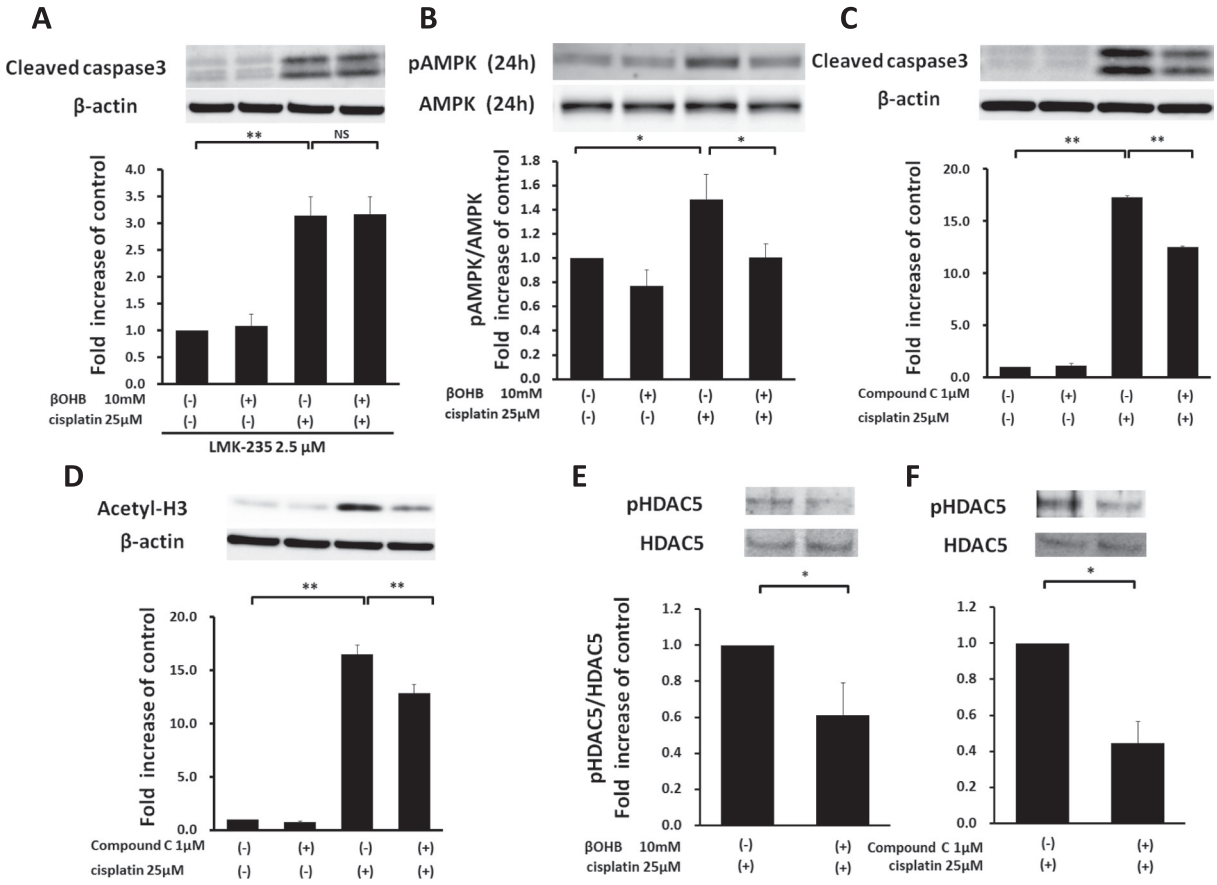

Fig. 5. βOHB reduces cisplatin-induced nephrotoxicity via the AMPK signaling pathway in HRCE cells. (A) HRCE cells were treated with vehicle or cisplatin (25 μM) with or without βOHB (10 mM) in the presence of LMK-235, a selective HDAC4/5 inhibitor (2.5 μM) for 48 h. Cell lysates were analyzed by western blot for cleaved caspase-3. The cleaved caspase-3 level relative to β-actin in control cells was set to 1.0. (B) HRCE cells were treated with vehicle or cisplatin (25 μM) with or without βOHB (10 mM) for 24 h. Cell lysates were analyzed by western blot for phospho-AMPK and AMPK. The phospho-AMPK level relative to AMPK in control cells was set to 1.0. (C) HRCE cells were treated with vehicle or cisplatin (25 μM) with or without Compound C (1 μM) for 48 h. Cell lysates were analyzed by western blot for cleaved caspase-3. The cleaved caspase-3 level relative to β-actin in control cells was set to 1.0. (D) HRCE cells were treated with vehicle or cisplatin (25 μM) with or without Compound C (1 μM) for 48 h. Cell lysates were analyzed by western blot for acetyl histone H3. The acetyl histone H3 level relative to β-actin in control cells was set to 1.0. (E, F) HRCE cells were treated with cisplatin (25 μM) with or without βOHB (10 mM) or with cisplatin (25 μM) with or without Compound C (1 μM) for 48 h. Cell lysates were analyzed by western blot for phospho-HDAC5 and HDAC5. The phospho-HDAC5 level relative to HDAC5 in cells treated with cisplatin was set to 1.0. Data are expressed as the mean ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, NS not significant by one-way ANOVA with Tukey-Kramer multiple comparison test (A–D) and by Student *t*-test (E, F).

suggested that the necroptotic pathway was stimulated by the high dose of cisplatin but not by the low dose. This is concordant with a previous report showing that low concentrations of cisplatin induced apoptosis and that high concentrations of cisplatin (200–800 μM) induced necrosis in primary cultures of mouse proximal tubular cells [21]. The necroptotic pathway is, thus, unlikely to be involved in our experimental system with cisplatin at 25 μM. Taken together, these results show that βOHB reduces cisplatin-mediated activation of some caspases, including caspase-3 and -9, at least in part via an intrinsic apoptotic pathway related to DNA damage.

3.3. Effects of βOHB combined with cisplatin on apoptosis of HRCE cells

Next, we examined whether βOHB actually reduced the cell apoptosis of HRCE cells induced by cisplatin. HRCE cells were treated with cisplatin, βOHB, or both for 48 h and stained with Annexin V-FITC and PI. Apoptosis was quantified using flow cytometry. Because a necrotic pathway is unlikely to participate in the cisplatin-induced cell death in our experimental system as mentioned in the previous paragraph, we focused in apoptotic findings. Annexin V-positive/PI-negative cells were identified as cells in the early stages of apoptosis, while Annexin V-positive/PI-positive cells were identified as cells in the late stage of apoptosis. The percentage of cells that were Annexin V-positive were represented as the portion of apoptotic cells. The apoptotic rate at 48 h was significantly lower in the treatment of these cells with βOHB + cisplatin than that with cisplatin alone (Fig. 3). Thus, the treatment with βOHB reduced apoptosis induced by cisplatin in HRCE cells.

3.4. βOHB reduces cisplatin-induced apoptosis by modifying HDAC in HRCE cells

A previous report showed that βOHB inhibits endogenous HDACs [5]. Therefore, we examined whether βOHB affected cisplatin-induced apoptosis through a HDAC-associated pathway. Cisplatin alone significantly increased acetylation of histone H3 by 22-fold compared to control, and βOHB significantly reduced the cisplatin-induced acetylation of histone H3 at 10 mM (Fig. 4A). Furthermore, treatment with cisplatin alone significantly reduced the protein levels of HDAC4/5/6, and βOHB significantly attenuated the cisplatin-mediated reduction of HDAC4/5/6 (Fig. 4B). Treatment with βOHB alone did not significantly change the protein levels of HDAC4/5/6 (Fig. 4B). The reduction of cisplatin-induced acetylation of histone H3 by βOHB was completely blocked by TSA, which is a pan-HDAC inhibitor (Fig. 4C), and reduction

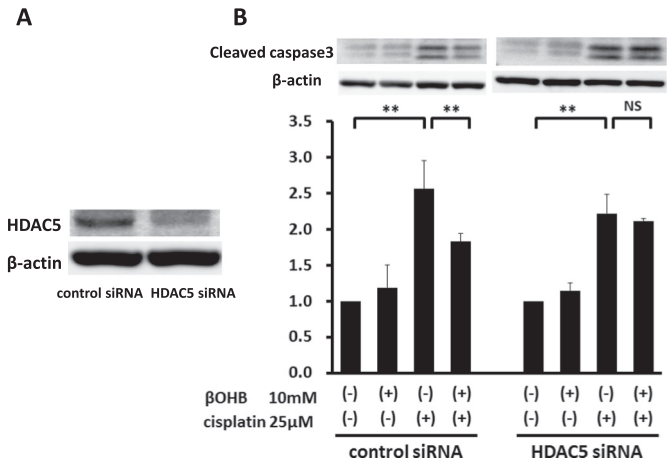

Fig. 6. βOHB reduces cisplatin-induced nephrotoxicity via activation of HDAC5 in HRCE cells. (A) Representative immunoblot of HDAC5 protein in siRNA-mediated knockdown cells. (B) HRCE cells were treated with vehicle or cisplatin (25 μM) with or without βOHB (10 mM) in cells treated with control or HDAC5 siRNA for 48 h. Cell lysates were analyzed by western blot for cleaved caspase-3. The cleaved caspase-3 level relative to β-actin in control cells was set to 1.0. Data are expressed as the mean ± SD of three independent experiments. $^{**}P < 0.01$, NS not significant by one-way ANOVA with a Tukey-Kramer multiple comparison test.

of caspase-3 cleavage by βOHB was completely blocked by the treatment with TSA (Fig. 4D). Taken together, these results show that βOHB reduces activation of caspase-3 by cisplatin, at least in part via a HDAC-mediated pathway.

3.5. βOHB reduces cisplatin-induced nephrotoxicity via the AMPK signaling pathway

To investigate the molecular mechanism of the protective effect of βOHB against cisplatin-induced nephrotoxicity, we examined which HDACs participate in the reduction of histone H3 acetylation by βOHB. The treatment with LMK-235, a selective inhibitor of HDAC4 and HDAC5, completely blocked the reduction of caspase-3 cleavage in HRCE cells (Fig. 5A). A recent study showed that AMP-activated kinase (AMPK) regulates the activation of HDAC5 [23]. To examine this possibility, immunoblotting of phosphorylated AMPK was performed in HRCE cells. Cisplatin induced phosphorylation of AMPK significantly compared to control, and the treatment with βOHB + cisplatin decreased the phosphorylation of AMPK significantly compared to cisplatin alone (Fig. 5B). Furthermore, the treatment with cisplatin + Compound C, a selective inhibitor of AMPK, significantly reduced the cleavage of caspase-3 and the acetylation of histone H3 compared to cisplatin alone in HRCE cells (Fig. 5C, D). Phosphorylated AMPK can phosphorylate HDAC5, which facilitates HDAC5 export from the nuclear to cytoplasmic compartment. The treatment with acadesine, an AMPK activator, also increases acetylation of histone H3 [24]. The phosphorylated/non-phosphorylated HDAC5 ratio with βOHB + cisplatin or Compound C + cisplatin was significantly lower than that with cisplatin alone in HRCE cells (Fig. 5E, F). Taken together, these results show that βOHB reduces cisplatin-induced apoptosis of HRCE cells, at least in part through the AMPK signaling pathway.

3.6. βOHB reduces cisplatin-induced nephrotoxicity via modulation of HDAC5

Lastly, we performed HDAC5 gene silencing in HRCE cells using siRNA to examine whether βOHB-mediated suppression of caspase-3 cleavage was dependent on HDAC5. Under the condition of HDAC5 gene silencing (Fig. 6A), the βOHB-induced suppression of cisplatin-induced cleavage of caspase-3 was cancelled (Fig. 6B). Taken together, these results show that βOHB attenuates cisplatin-induced apoptosis of HRCEs, at least in part via modification of HDAC5. (See Fig. 7.)

4. Discussion

In this study, we showed that βOHB, a ketone body, reduces

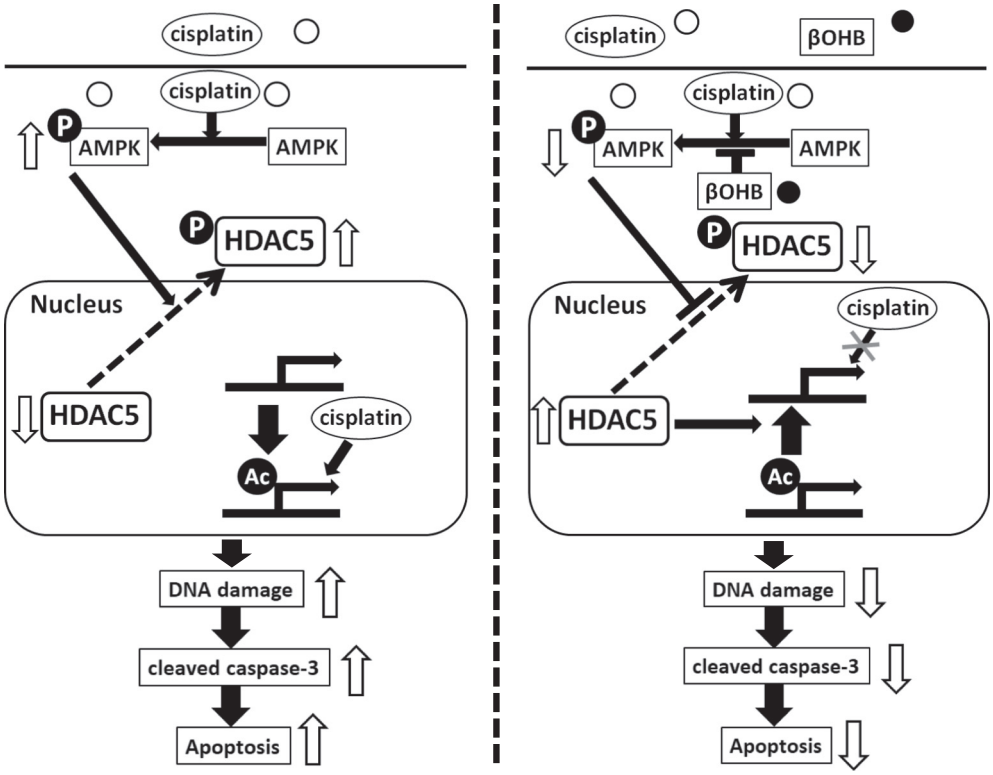

Fig. 7. Model showing how βOHB may reduce the cytotoxic effect of cisplatin via activation of HDAC5 in human renal cortical epithelial cells. βOHB increases expression of HDAC5 and retains HDAC5 in the nucleus through decreasing phosphorylated AMPK, resulting in tightening of chromatin due to deacetylation of histones.

cisplatin-induced nephrotoxicity via activation of HDAC5. To the best of our knowledge, this is the first report to suggest potential reduction of cytotoxicity of cisplatin chemotherapy using βOHB.

βOHB is a metabolic intermediate that constitutes about 70% of ketone bodies produced in liver, mainly from oxidation of fatty acids released from adipose tissue [25]. βOHB is transported by blood to peripheral tissues, where it is oxidized via the TCA cycle to provide the energy required by tissues such as skeletal and heart muscle, and renal cortex [26]. Mild elevation of blood ketone bodies occurs in the process of normal aging [27] and in congestive heart failure [28–30]. However, it remains unclear whether this elevation reflects an adaptive mechanism to maintain cell metabolism or contributes to progression of diseases.

A key finding in the current study is that βOHB reduced cisplatin-induced apoptosis by modulating HDAC5, an epigenetic modifier, via the AMPK pathway. AMPK is a cellular metabolic sensor that plays an important role in control of energy homeostasis in response to external stresses [31,32]. A recent study showed that βOHB increased production of ATP and inhibited AMPK phosphorylation in hypothalamic N-38 cells [33]. Another report showed that βOHB decreased AMPK phosphorylation in hypothalamic GT1–7 cells under physiological conditions [34]. Phosphorylated AMPK phosphorylates HDAC5 at Ser259 and Ser498, a promoting the export of HDAC5 from nucleus to cytoplasm. The treatment with acadesine, an activator of AMPK, increases the acetylation level at the H3K9 and H3K14 sites [24].

In the current study, βOHB significantly reduced cisplatin-induced phosphorylation of AMPK in HRCE cells, and HDAC5 silencing in these cells completely cancelled the βOHB-mediated suppression of the cisplatin-induced cleavage of caspase-3. Based on these findings, the renoprotective effects of βOHB are at least partly dependent on the HDAC5 pathway and involve the reduction of AMPK phosphorylation. βOHB inhibits HDAC1, 3 and 4 (classes I and IIa) in HEK293 cells under normal conditions, and administration of exogenous βOHB in fasting or calorie-restrictive conditions increases histone acetylation in mouse tissues [5]. However, the increase in acetylation of H3 induced by βOHB alone was much less than that induced by cisplatin, suggesting that these acetylations are under discrete regulation by βOHB alone and by cisplatin without or with βOHB. Thus, βOHB suppressed the increase in acetylation induced by cisplatin, and its action exceeded the direct action of βOHB in the absence of cisplatin. Therefore, we speculate that the protective effects of βOHB as an inhibitor or an activator of HDACs may depend on experimental conditions and the type of HDAC upon which βOHB acts.

The βOHB-related molecular mechanism downstream of HDAC5 is unclear in HRCE cells. A tight chromatin structure maintained by HDAC activity prevents cisplatin from approaching DNA, and relaxation of chromatin by HDAC inhibition increases accessibility of cisplatin to DNA [35]. In the current study, treatment with βOHB + cisplatin significantly increased the protein levels of HDAC4, 5 and 6 in HRCE cells, compared to cisplatin alone. The phosphorylated/non-phosphorylated HDAC5 ratio with βOHB + cisplatin was also significantly lower than that with cisplatin alone. Therefore, we postulate that βOHB increases expression of HDAC4/5/6 and retains HDAC5 in the nucleus through decreasing phosphorylated AMPK, resulting in tightening of chromatin due to deacetylation of histones with keeping cisplatin away from DNA.

5. Conclusions

βOHB attenuated cisplatin-induced apoptosis by activation of HDAC5 in HRCE cells (Fig. 6). Our data suggest that βOHB may be a new therapeutic agent that mitigates cisplatin nephropathy. Finally, a selective HDAC5 agonist might also serve as an agent to ameliorate renal tubular cell death in cisplatin treatment.

Abbreviations

βOHB	β-hydroxybutyrate
HEK293	human embryonic kidney 293
HRCE	human renal cortical epithelial
HDAC	histone deacetylase
SGLT2	Sodium glucose co-transporter 2
AKI	acute kidney injury
CKD	chronic kidney disease
AMPK	AMP-activated kinase
TSA	trichostatin
RIP3	receptor-interacting protein 3
BUN	blood urea nitrogen

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Conflict of interest

The authors have no conflict of interest.

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