Original Research

Corilagin alleviates intestinal ischemia/reperfusion injury by relieving oxidative stress and apoptosis via AMPK/Sirt1-autophagy pathway

Bin Li[1,2]*, Wenlian Li[1]*, Meiling Zheng[1], Yunxiang Wang[1], Yunpeng Diao[1,2], Xiaojuan Mou[1] and Jing Liu[1,2]

[1]College of Pharmacy, Dalian Medical University, Dalian 116044, China; [2]Dalian Anti-Infective Traditional Chinese Medicine Development Engineering Technology Research Center, Dalian 116044, China
*These authors contributed equally to this paper.
Corresponding authors: Yunpeng Diao. Email: diaoyp@163.com; Jing Liu. Email: liujing8166@163.com

Impact Statement

Our study unravels for the first time that Corilagin, one of the representative components of the chebuloids, has a significant protective effect against pathological injury induced by intestinal ischemia-reperfusion, which is mainly reflected in the alleviation of oxidative stress, apoptosis, and intestinal barrier damage. We also found that Corilagin can activate autophagic flow through AMPK/Sirt1 pathway, which may be one of the mechanisms for Corilagin's therapeutic effects. In conclusion, our study further contributes to the usage and potential mechanisms of Corilagin.

Abstract

Intestinal ischemia/reperfusion (II/R) injury is a common pathological process with high clinical morbidity and mortality. Autophagy plays an important role in the pathological development of II/R. Corilagin (CA) is a natural ellagitannin with various pharmacological effects such as autophagy regulation, antioxidant, and antiapoptosis. However, whether CA alleviates II/R injury is still unclear. In this study, we had found that CA significantly attenuated II/R induced intestinal tissue pathological damage, oxidative stress, and cell apoptosis in rats. Further studies showed that CA significantly promoted AMPK phosphorylation and sirt1 expression, and thus activated autophagy by upregulating protein expression of autophagy-related proteins Beclin1 and LC3II and promoting SQSTM1/P62 degradation both *in vivo* and *in vitro*. Inhibition of AMPK phosphorylation by its inhibitor compound C(CC) significantly abolished CA-mediated autophagy activation and the relievable effects on oxidative stress and apoptosis *in vitro*, suggesting the excellent protective activity of CA against II/R injury via AMPK/Sirt1-autophagy pathway. These findings confirmed the potent effects of CA against II/R injury, and provided novel insights into the mechanisms of the compound as a potential candidate for the treatment of II/R.

Keywords: Intestinal ischemia-reperfusion, corilagin, autophagy, AMPK/Sirt1

***Experimental Biology and Medicine* 2023; 248: 317–326. DOI: 10.1177/15353702221147560**

Introduction

Intestinal ischemia/reperfusion (II/R) is an inevitable complication following various serious diseases, such as severe trauma, traumatic shock, severe infection, intestinal obstruction, and surgery.[1,2] It is one of the critical clinical challenges with high morbidity and mortality.[3] Redirecting the blocked blood flow to intestine after ischemia is essential for the repair of damaged intestine, but also triggers a cascade of additional organ damages frequently exceeding the original ischemic insult, namely, reperfusion injury.[4] Ischemia/reperfusion (I/R) injury is caused by multiple factors, including oxygen radical formation, neutrophil accumulation, excessive inflammatory cytokine release, and cell apoptosis.[5] II/R usually leads to increased intestinal permeability and disruption of mucosal barrier function, which may trigger intestinal infections and even systemic inflammatory responses and distal organ failure.[6,7] Therefore, innovative therapeutic strategies are needed to mitigate II/R-induced intestinal damage.

Autophagy, a tightly regulated intracellular catabolic process that is involved in the disposal of damaged organelles and dysfunctional proteins through autophagosomal sequestration and subsequent lysosomal degradation, plays crucial roles in general homeostasis through regulating inflammation, oxidative stress, and apoptosis in diverse diseases including II/R injuries.[8,9] Defective autophagy usually potentiates pro-inflammatory cytokine production and promotes damaged cytoplasmic components accumulation in some ischemic diseases, resulting in excessive inflammation

response, oxidative stress, and apoptosis.[10] And, autophagy activation could significantly ameliorate ischemic injuries by direct disposal of dysfunctional intracellular components and aggregated inflammasome structures, thus decreasing cell death and inflammation response.[11,12] So, appropriate autophagy activation might serve as a viable II/R therapeutic strategy.

Multiple signal pathways are involved in autophagy regulation including AMPK/Sirt1, PI3K/AKT/mTOR, MEK/ERK and HIF-1α/BNIP3 signaling pathways.[13–16] Both AMPK and Sirt1 are fuel-sensing molecules, which are closely related to homeostasis and energy balance in the body.[17] Being phosphorylated at Thr172 activates AMPK; then the activated AMPK further activates Sirt1 and regulates inflammation, oxidative stress, and apoptosis in a Sirt1-dependent manner.[18,19] Increasing evidence has indicated that AMPK/Sirt-1 pathway linked autophagy plays a protective effect in various diseases including I/R injuries.[13] Various natural products are observed to protect against multiple diseases by regulating AMPK/Sirt1/autophagy signaling pathway. For instance, resveratrol has been found to attenuate early brain injury after subarachnoid hemorrhage and bone cancer pain by regulating autophagy via AMPK/Sirt1 pathway.[20,21] However, the role of the AMPK/Sirt1/autophagy pathway in II/R-induced intestinal damage is still unknown.

Corilagin (CA, Figure 1(a)), a natural ellagitannin found in a variety of plants, has been shown to possess many biological and pharmacological properties, such as antioxidant, antiinflammatory, and antiapoptosis activities.[22–24] CA has been reported to improve ischemic brain injury in rats by reducing oxidative stress and promoting angiogenesis, and protect against I/R-induced acute lung injury by improving apoptotic pathways.[22–24] However, no studies have reported the effects and molecular mechanisms of CA in alleviating II/R-induced intestinal damage. Recent studies have shown that CA could ameliorate oxidative stress and restore autophagic flux to alleviate nonalcoholic fatty liver disease[25] and promote AMPK phosphorylation to activate its downstream signaling pathway to alleviate acetaminophen-induced liver injury, suggesting the possibility of CA regulating autophagy by promoting AMPK phosphorylation.[26] Therefore, we proposed that CA might effectively ameliorate II/R injury by restoring II/R-impaired autophagy flux through activating AMPK/Sirt1 signaling pathway. Both *in vivo* and *in vitro* studies were carried out to verify our proposal.

Materials and methods

Drugs and reagents

CA (purity quotient: ⩾98%), purchased from Shanghai Source Leaf Biological Technology Co. Ltd (Shanghai, China). Reagent kits for detection of diamine oxidase (DAO), myeloperoxidase (MPO), malondialdehyde (MDA), and glutathione (GSH) were obtained from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Dulbecco's minimum essential medium (DMEM) and fetal bovine serum (FBS) were purchased from ScienCell research laboratories (CA, USA). Terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick-end labeling (TUNEL) kit was provided by Roche Company (Roche, Shanghai, China). Reactive oxygen species (ROS) Assay Kit was purchased from Beyotime Biotechnology (Shanghai, China). Cell counting kit-8 (CCK-8) kit was provided by Biotool (Shanghai, China). Compound C dihydrochloride (CC, 99.91% pure) was purchased from MCE (USA).

Animal husbandry and II/R rat model establishment

Healthy male Sprague–Dawley rats weighting 180–220 g were obtained from Animal Center of Dalian Medical University (Dalian, China) (Certificate of Conformity: No. SYXK (Liao) 2018-0001). All the animal experiments were performed according to National Institutes of Health Guide for the use of animals in laboratory experiments and approved by Dalian Medical University Animal Care and Ethics Committee (approval number: AEE19007). All rats were adaptively fed for one week before use in a controlled laboratory conditions with a temperature of 24 ± 2°C, humidity of 60 ± 10%, and 12/12 h light/dark cycle.

Rats were fasted for 12 h with free access to water before the experiments. The rat II/R injury model was established according to the previous study.[27] Rats were anesthetized with 20% ethyl carbamate and fixed in the supine-face up. Then, isolated superior mesenteric artery (SMA), occluded SMA with an atraumatic arterial clamp for 1 h, and then gently removed the clamp for 2 h reperfusion. After reperfusion, blood samples were taken from abdominal aorta, then rats were sacrificed and the whole small intestine was removed and washed with normal saline. All samples were stored at −80°C for determination.

The rats were randomly divided into five groups ($n = 6$ in each group): (1) Sham group (Sham): rats were administered vehicle intragastrically for three days and underwent isolation of SMA without occlusion; (2) II/R group: rats were administered vehicle intragastrically for three days and subjected to II/R injury. (3) CA (25 mg/kg) + II/R group: rats were intragastric administrated with CA (25 mg/kg) once daily for three consecutive days prior to II/R induction. (4) CA (50 mg/kg) + II/R group: rats were intragastric administrated with CA (50 mg/kg) once daily for three consecutive days prior to II/R induction. (5) CA (50 mg/kg) + Sham group: rats were intragastric administrated with CA (50 mg/kg) once daily for three consecutive days before sham operation.

Approximately 2 cm of isolated intestine was washed with ice-cold normal saline, fixed with 4% paraformaldehyde solution for at least 24 h, and further used for hematoxylin-eosin (H&E), immunohistochemical (IHC) and the terminal deoxynucleotidyl transferase-mediated biotinylated deoxyuridine triphosphate nick-end labeling (TUNEL) staining. Serum was used to measure the levels of diamine oxidase (DAO) and the supernatant of rat intestinal homogenate was used to measure myeloperoxidase (MPO), malondialdehyde (MDA), and glutathione (GSH) levels.

Cell culture

Human colorectal adenocarcinoma cell line Caco-2 was used to establish the hypoxia-reoxygenation (H/R) injury model in this study. Caco-2 cells were cultured in DMEM

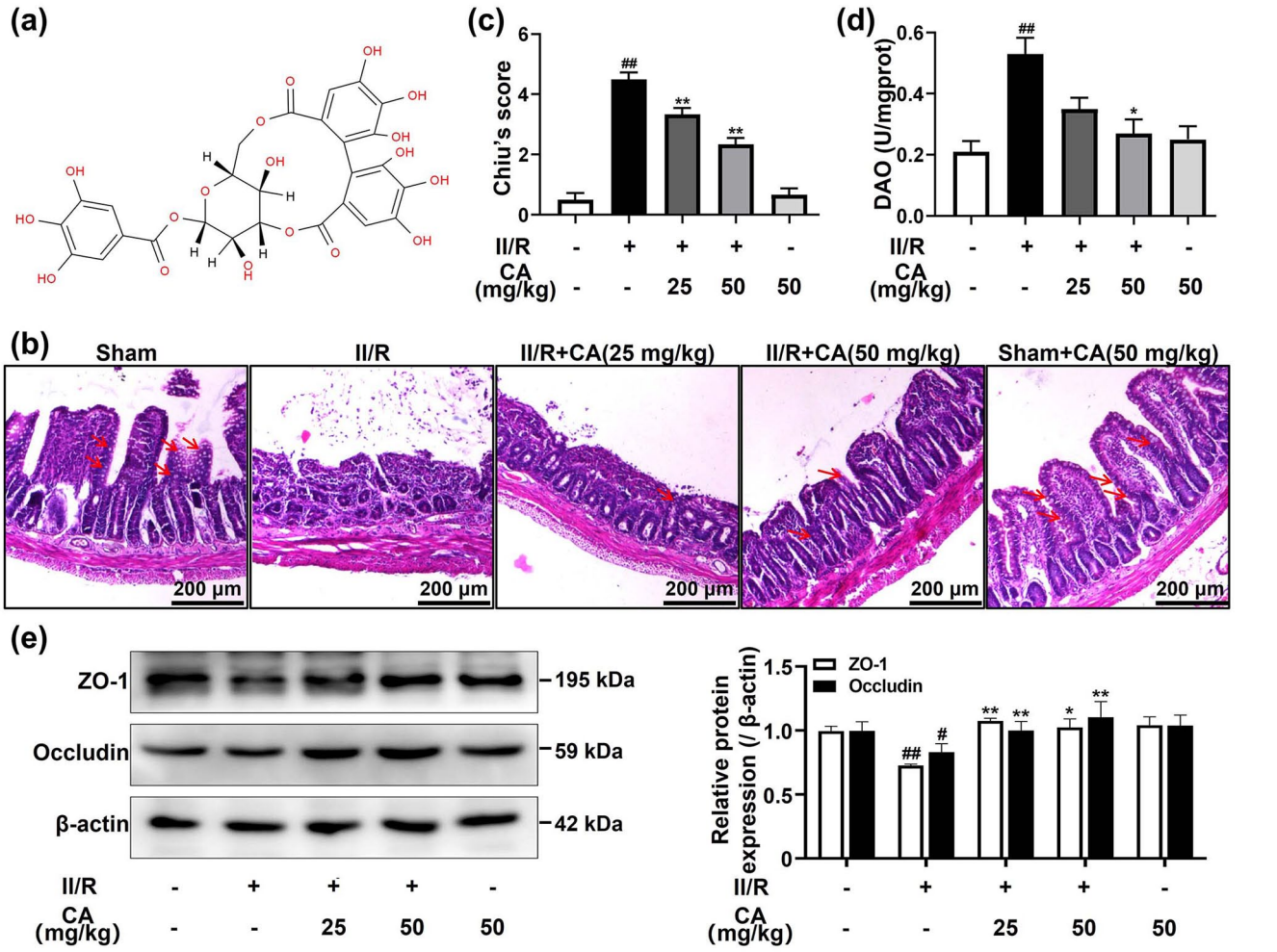

Figure 1. CA ameliorated intestinal ischemia/reperfusion (II/R)-induced intestinal damage in rats. (a) The chemical structure of CA. (b) Representative images of intestinal histology (scale bar = 200 μm). Goblet cells were indicated by red arrows. (c) Histopathological scores (Chiu's scores) of the intestine. (d) The levels of serum DAO ($n \geqslant 3$). (e) The expression levels of occludin and ZO-1 proteins ($n \geqslant 3$).
Data are expressed as the mean ± SEM.
$^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus sham group, $^{*}P < 0.05$ and $^{**}P < 0.01$ versus II/R group.

supplemented with 10% FBS in a humidified atmosphere of 5% CO_2 at 37°C. To establish H/R model, the cells were incubated in low-glucose DMEM and transferred to a microaerophilic system containing 5% CO_2, 1% O_2 and balanced with 94% N_2 for 12 h at 37°C, then cultured in normal conditions for 12 h reoxygenation. The changes of cell viability under different reoxygenation times (0, 3, 6, 12, and 24 h), different concentrations of CA (0, 1, 5, 10, 20, 50, 100, 200, and 300 μg/mL) for 12, 24 h and pretreated with CA at different concentrations after H/R injury for 6, 12, and 24 h were measured by the CCK-8 kit, respectively.

Intracellular ROS were measured with flow cytometry analysis according to manufacturer's instructions. Cellular apoptosis was detected by the Hoechest 33342 kit according to manufacturer's instructions.

Western blotting analysis

Total protein samples extracted from Caco-2 cells or rat intestinal tissues were separated using SDS-PAGE (6–15%) and transferred to PVDF membranes. Then, the membranes were blocked by 5% defatted milk for 2 h at room temperature, incubated with the following primary antibodies: AMPK, P-AMPK (Cell Signaling Technology, MA, USA), Sirt1 (Bioss, Beijing, China), P62, LC3II/I, Beclin1, Bax, ZO-1, Occludin, and Bcl-2 (Proteintech, Wuhan, China) overnight at 4°C, and then individually incubated with the corresponding secondary antibodies for 2 h at indoor temperature. After extensive washing by Tris buffered solution with 0.1% tween 20 (TBST), the membranes were exposed to ECL detection system. Protein's quantification was determined in optical density units using Image Lab software (Bio-Rad, CA, USA) and normalized to the corresponding sample protein expression of β-actin.

Statistical analysis

The animal experiments, *in vitro* experiments, and data analyses were conducted according to a single-blind study design. Data was compared between three or more groups using one-way analysis of variance (ANOVA), and between two groups using Student's *t*-test. Data were expressed as the means ± standard error of the mean. Data were normally distributed, and each group showed similar variances.

All experiments were repeated at least three times and a *P*-value of <0.05 was considered statistically significant.

Results

CA ameliorated II/R-induced intestinal damage

To determine whether CA exerts protective effects, intestinal morphologic alterations were measured by H&E staining. The jejunal mucosal epithelium in the sham group or CA-pretreated sham group was intact with clearly visible goblet cells. Compared with the sham rats, II/R induced significant intestinal morphological changes with massive inflammatory cell infiltration accompanied by denuded villi, dilated capillaries, digestion as well as disintegration of lamina propria, hemorrhage, and ulceration. The II/R-induced intestinal morphologic alterations were significantly attenuated by CA pretreatment (25 and 50 mg/kg) in a dose-dependent manner with Chiu's scores markedly restored (Figure 1(b) and (c)). The histopathological analysis suggested the protective effects of the CA pretreatment against II/R-induced intestinal epithelial injury.

CA ameliorated II/R-injured intestinal barrier

The DAO levels in serum and the expression levels of tight junction proteins ZO-1 and occludin were examined to assess the degree of intestinal epithelial barrier damage. Compared with the sham group, the serum DAO level was significantly increased in II/R injured rats, and CA pretreatment (25 and 50 mg/kg) significantly reversed the increased serum DAO levels of II/R rats in a dose-dependent manner (Figure 1(d)). Western blotting analysis showed that the expression levels of both occludin and ZO-1 were reduced in II/R group compared with sham group, and CA pretreatment significantly increased the dysregulated protein expression in II/R group (Figure 1(e)). These results demonstrated the protective effects of CA on II/R-injured intestinal barrier.

CA mitigated II/R-induced oxidative stress and apoptosis

Excessive oxidative stress and epithelial apoptosis are critical features in the pathogenesis of II/R injury.[28] The increased levels of MDA (a biomarker of oxidative damage) and downregulation of GSH (biomarkers of antioxidant capacity) were observed in II/R-injured group compared with the sham group, indicating the serious oxidative stress induced by II/R. The levels of MPO, which is a biomarker of neutrophil infiltration, were also increased in II/R injured rats. In contrast, CA pretreatment (25 and 50 mg/kg) significantly mitigated the dysregulated expression levels of MDA, GSH, and MPO in II/R injured rats in a dose-dependent manner (Figure 2(a) to (c)), showing the effective alleviation effects of CA on II/R induced oxidative stress.

In mammals, the Bcl-2 family proteins play an important role in the process of apoptosis regulation. The Bax/Bcl-2 ratio determines the trend of cell apoptosis.[29] The upregulation of Bax/Bcl-2 ratio was observed by western blotting analysis, showing excessive cell apoptosis during II/R injury. CA pretreatment (25 and 50 mg/kg) significantly alleviated the dysregulated expression of Bax/Bcl-2 ratio in II/R injury (Figure 2(d)). Furthermore, TUNEL-positive cells were rarely observed in the sham rats and CA-pretreated sham rats. The number of TUNEL-positive cells was increased in the intestinal villus during II/R injury, and significantly decreased by CA pretreatment in a dose-dependent manner (Figure 2(e)). The above results have demonstrated the protective effects of CA on II/R induced epithelial apoptosis.

CA restored impaired autophagy activity and activated AMPK/Sirt1 signaling pathway in II/R injured rates

II/R injury induced significant downregulation of protein expression levels of Beclin-1 (a key regulator in autophagosome formation) and lapidated LC3 (LC3II) (a hallmark of autophagy activation) and decreased the degradation of classic autophagy substrate SQSTM1/p62 (Figure 3), indicating the impaired autophagy during II/R injury. CA pretreatment significantly restored the impaired autophagy activity in II/R rats (Figure 3).

AMPK/Sirt1 pathway is involved in regulating autophagy in diverse injuries.[20] AMPK is activated by phosphorylating the Thr172 residue on its α-subunit by upstream kinases.[19] Western blotting analyses showed that the expression ratio of p-AMPK/AMPK was significantly decreased by II/R injury and dramatically rebounded by CA pretreatment (Figure 3(b)). The protein expression level of Sirt1 showed a similar trend to the p-AMPK/AMPK ratio (Figure 3(b)).

CA ameliorated H/R induced cell damage through AMPK/Sirt1/autophagy pathway

To investigate the role of the AMPK/Sirt1/autophagy pathway in the protective effects of CA on II/R-induced injury, Caco-2 cells were employed and challenged with H/R to mimic the circumstance *in vitro*. The effects of CA on cell viability in normal conditions were first investigated using Caco-2 cells pretreated with CA at the concentration ranges of 2.5, 5, 10, 20, 50, 100, 200, and 300 μg/mL for 24 h to screen out appropriate concentrations of CA. The CCK-8 assay showed that CA did not exert significant effects on the cell viability of Caco-2 under normal conditions with CA concentrations less than 20 μg/mL (Figure 4(a)). CA pretreatment at concentration ranges of 2.5, 5, 10, and 20 μg/mL on H/R-injured Caco-2 cells for 12 h significantly restored the cell viability decrease induced by H/R injury (Figure 4(b)). Those results have documented the low cytotoxicity and effective anti-H/R injury ability of CA.

Western blotting analyses showed the downregulation of expression levels for Beclin-1 and lapidated LC3 (LC3II) and the inhibition of classic autophagy substrate SQSTM1/p62 degradation in H/R-injured cells (Figure 5(a)), indicating impaired autophagy during H/R injury. The downregulation of expression ratio of p-AMPK/AMPK and Sirt1 expression levels were also observed (Figure 5(a)), indicating the inactivation of AMPK/Sirt1 pathway. CA pretreatment significantly restored the impaired autophagy activity and AMPK/Sirt1 pathway in H/R injured cells. But the amelioration of CA was neutralized by compound C (CC, also

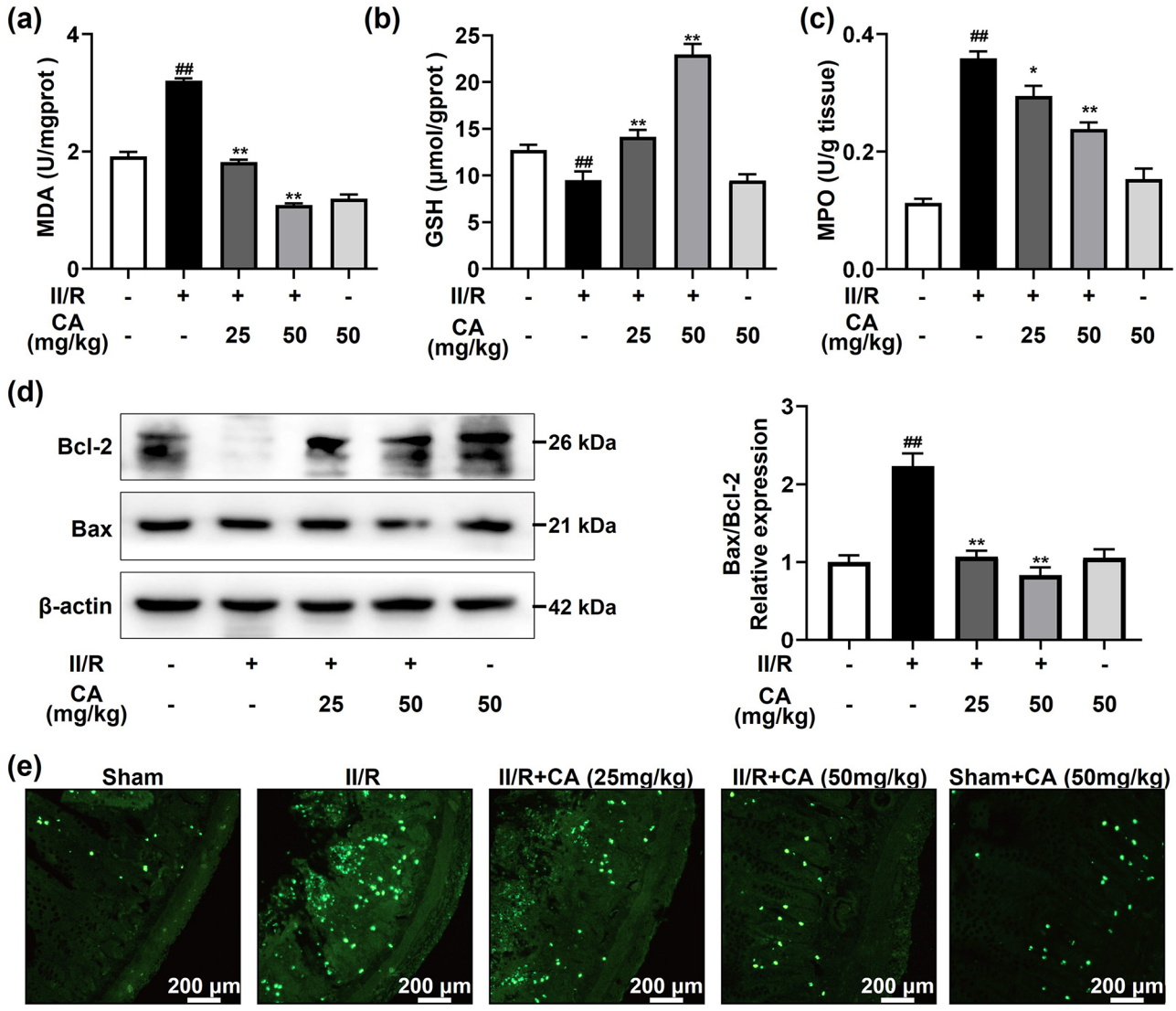

Figure 2. CA suppressed II/R-induced oxidative stress and epithelial apoptosis. (a to c) Effects of CA on MDA, GSH and MPO in intestines ($n \geqslant 3$). (d) Western blotting analysis of protein expression levels of Bcl-2 and Bax in intestine ($n \geqslant 3$). Values in the sham group are set to 1, and other values are given relative to the sham group. (e) TUNEL staining of each group (scale bar = 200 μm).
All values are expressed as the mean ± SEM.
$^{\#\#}P < 0.01$ versus sham group, $^{*}P < 0.05$ and $^{**}P < 0.01$ versus II/R group.

called dorsomorphin), the inhibitor of AMPK (Figure 5(a)). Furthermore, the effects of CA on H/R-induced cell apoptosis and oxidative stress were investigated. Similar to the results *in vivo*, H/R-induced significant cell apoptosis and oxidative stress while CA pretreatment exhibited protective effects on H/R-induced cell apoptosis and oxidative stress in Caco-2 cells (Figure 5). The protective effects of CA were counteracted by compound C (Figure 5). Thus, CA might ameliorate II/R-induced intestinal injury through the AMPK/Sirt1/autophagy pathway (Figure 6).

Discussion

II/R injury is a frequent clinical condition with high morbidity and mortality.[3] The development of innovative therapeutic strategies to mitigate II/R-induced intestinal damage is of great significance. CA is a natural ellagitannin in a variety of plants and has exhibited a series of biological and pharmacological properties such as antioxidant, antiinflammatory, antiapoptosis, and antitumor activities.[22,30] In this study, we examined the protective effects and possible mechanisms of CA on rat II/R injury. CA had shown significant amelioration of II/R-induced intestinal morphological damage, intestinal barrier dysfunction, oxidative stress, and cell apoptosis. To our knowledge, we may provide the first evidence of the protective effect of CA in II/R-induced intestinal injury, and the AMPK/Sirt1 activation-initiated autophagy may participate in CA's therapeutic effect on II/R injury.

Autophagy is a lysosome-mediated degradative pathway of cellular mechanisms for degrading misfolded proteins and is implicated in general homeostasis and multiple processes including cell differentiation and death.[28,31] LC3II, SQSTM1/P62, and Beclin1 are three critical regulators for autophagy execution. In this study, decreased expression of Beclin1 and

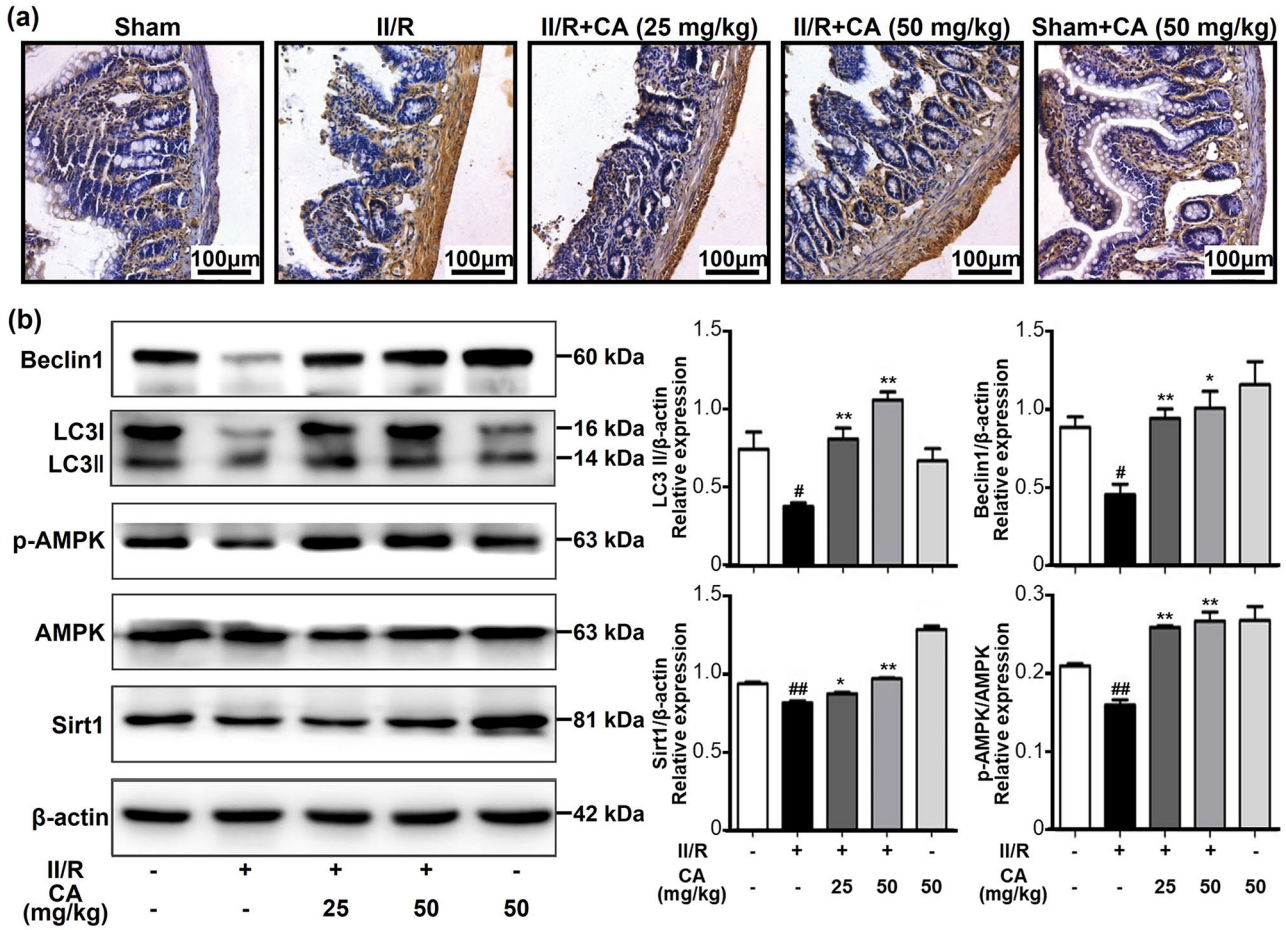

Figure 3. Effects of CA on autophagy and AMPK/Sirt1 signaling pathway. (a) Immunohistochemical staining of intestinal SQSTM1/P62 (scale bar = 100 μm). (b) Western blotting analysis of Beclin1, LC3II, AMPK, and phosphorylated AMPK in intestines ($n \geqslant 3$). Values in the sham group are set to 1, and other values are given relative to the Sham group.
All values are expressed as the mean ± SEM.
$^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus sham group, $^{*}P < 0.05$ and $^{**}P < 0.01$ versus II/R group.

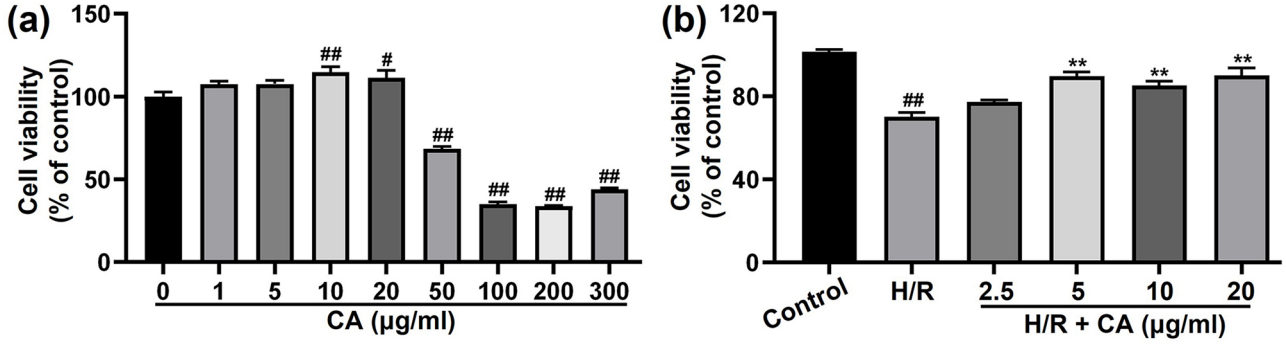

Figure 4. Protective effects of CA on H/R-induced Caco-2 cells injury. (a) Cytotoxicity of CA on Caco-2 cells. (b) Effects of CA on the Caco-2 cell viability under H/R condition.
All values are expressed as the mean ± SEM ($n \geqslant 3$).
$^{\#\#}P < 0.01$ versus control group, $^{*}P < 0.05$ and $^{**}P < 0.01$ versus H/R group.

LC3II and increase of SQSTM1/P62 were observed in II/R injury while CA restored this self-protective mechanism both *in vivo* and *in vitro*, suggesting the potential role of CA to prevent II/R-mediated autophagy impairment.

Autophagy plays a crucial role in II/R injury and is linked to oxidative stress and cell death, the two critical features in the pathogenesis of II/R injury. Usually, autophagy is activated as a feedback mechanism in the presence of oxidative stress and participates in ROS scavenging through degradation of dysfunctional mitochondria.[32] Autophagy also regulates apoptosis by degrading antiapoptotic factors or damaged molecules and organelles in mammalian cells.[33]

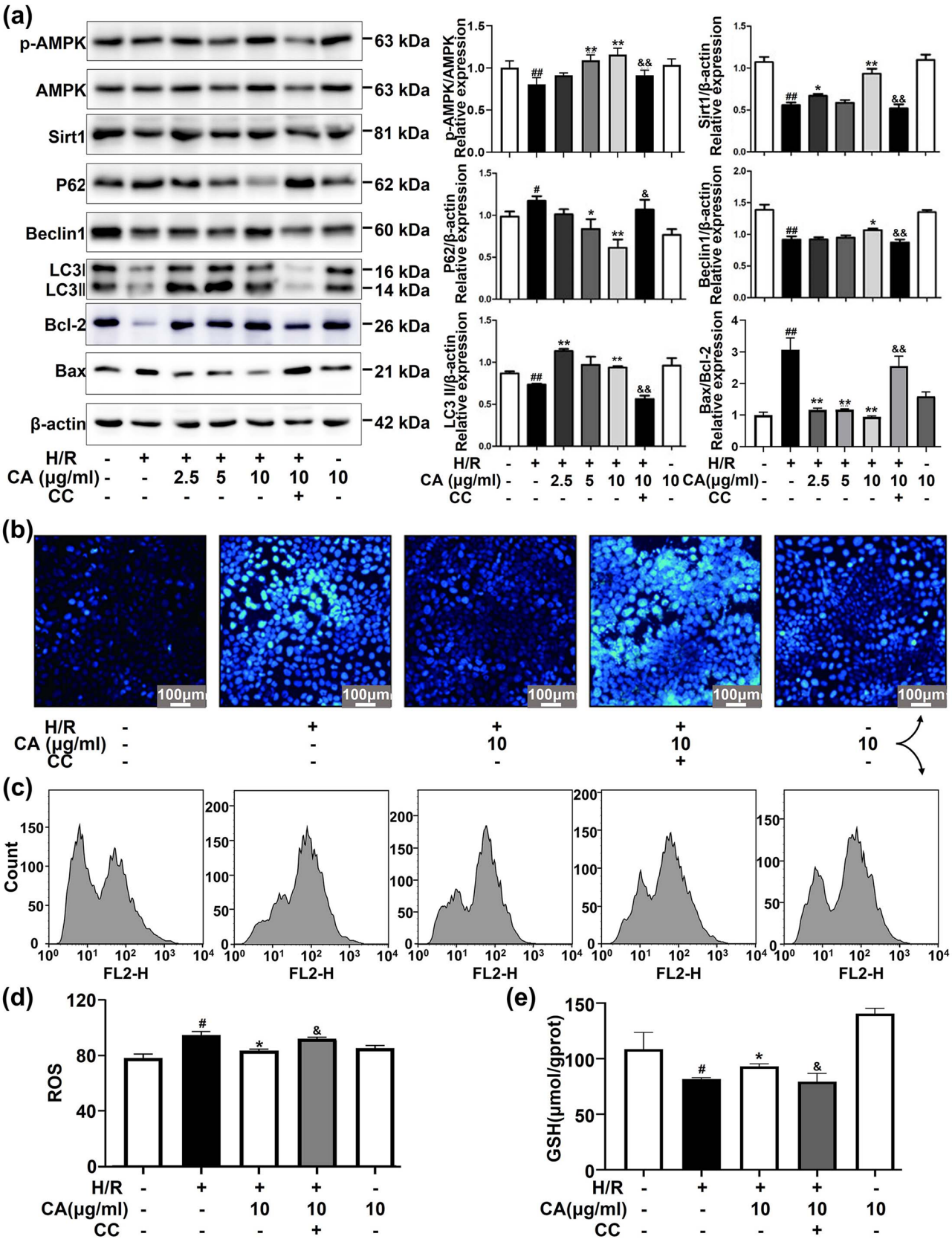

Figure 5. CA ameliorated H/R-induced intestinal injury through AMPK/Sirt1/autophagy pathway. (a) Protein expressions of p-AMPK, AMPK, Sirt1, SQSTM1/p62, Beclin1, LC3II, Bcl-2, and Bax by western blotting analysis. Values in the control group are set to 1, and other values are given relative to the control group. (b) Apoptosis determination of Caco-2 cells using Hoechest 33342 fluorochrome. (c and d) The level of intracellular reactive oxygen species (ROS) determined by flow cytometry. (e) The level of GSH.

All values are expressed as the mean ± ECM ($n \geqslant 3$).

$^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus control group, $^{*}P < 0.05$ and $^{**}P < 0.01$ versus H/R group; $^{\&}P < 0.05$ and $^{\&\&}P < 0.01$ versus CA (10 μg/mL) + H/R group.

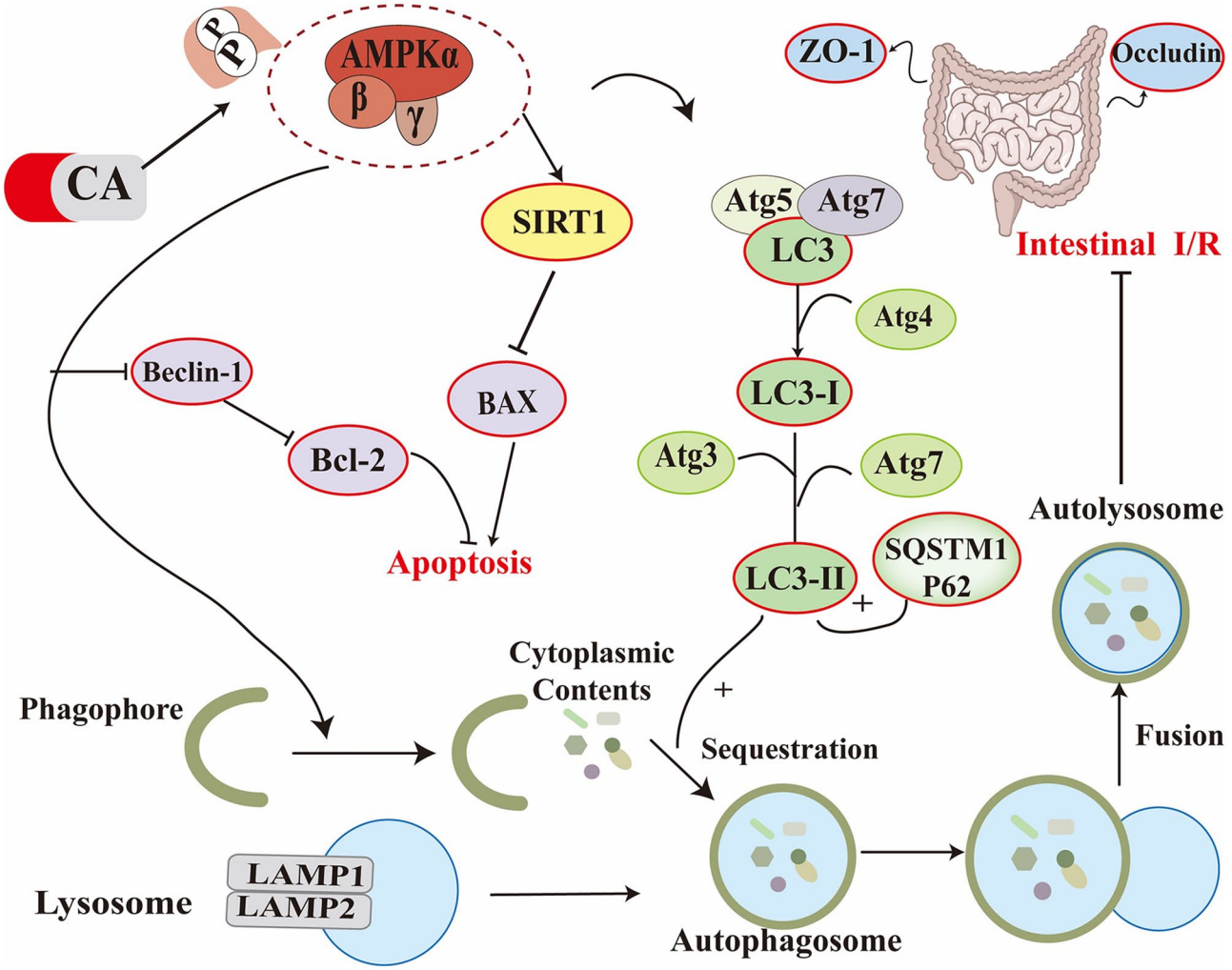

Figure 6. Schematic representation of the potential protective mechanism of CA in II/R-induced injury through the AMPK/Sirt-1/autophagy pathway.

Impaired autophagy is closely related to intestinal mucosal barrier damage with antimicrobial peptide secretion impairment and intestinal inflammation,[34] while autophagy activation served protective effects against II/R induced injury by inhibiting inflammation, oxidative stress, and apoptosis.[8] Our data showed that CA significantly reduced intracellular ROS levels, lipid peroxidation (MDA), and neutrophil infiltration (MPO) and increased the antioxidant capacity (GSH). CA pretreatment also significantly reversed apoptosis both *in vivo* and *in vitro* by inhibiting the activated apoptosis-related proteins (Bax/Bcl-2 ratio) and decreasing apoptotic positive cells. These results suggest the abirritation effect of CA on oxidative stress and cell apoptosis induced by II/R, which might closely relate to the activation of autophagy.

Autophagy can be regulated by several pathways, the most important of which is the AMPK/Sirt1 signaling pathway.[35] Sirt1 is a member of a highly conserved family of NAD-dependent deacetylases that regulate cellular energy and lifespan in mammals.[18] Sirt1 is generally activated to play its role in maintaining the energy balance when the body is under hypoxia or stress and its activity can be positively regulated by AMPK.[36,37] Srit1 is reported as an important regulator of autophagy by interacting with several essential components of the autophagy machinery and elevating autophagy, which can promote Beclin1 phosphorylation at Ser14, which is a key step for autophagy initiation.[38] AMPK/Sirt1-mediated autophagy is implicated in various diseases. Activation of AMPK/Sirt1 pathway is reported to restore impaired autophagy and inhibited inflammation reaction and apoptosis in acute pancreatitis, acute kidney injury, diabetic retinopathy and age-related mitochondrial dysfunction.[39–42] However, the role of AMPK/Sirt1-mediated autophagy is not clearly investigated in II/R. In this study, we found that a decrease in p-AMPK/AMPK and Sirt1 protein expression in II/R rats and CA pretreatment promoted AMPK phosphorylation and Sirt1 expression. This is parallel with autophagy levels but contradictory with oxidative stress and apoptotic levels. In addition, inhibition of AMPK activation by CC impaired the CA-induced upregulation of protein expression levels for p-AMPK, Sirt1, Baclin1, LC3II, and Bcl-2, the degradation of SQSTM1/p62 and Bax and the decrease of apoptotic cell number and intracellular ROS accumulation. These results confirmed that CA could significantly ameliorate II/R-induced intestinal damage, oxidative stress, and epithelial apoptosis through the AMPK/Sirt1/autophagy signaling pathway.

Conclusions

In conclusion, we showed here that CA mitigated II/R injury via inhibiting oxidative stress and apoptosis through promoting AMPK/Sirt1-mediated autophagy activity. CA might be a potential candidate for the treatment of II/R associated diseases, and this study provided experimental evidence for future clinical applications of CA.

AUTHORS' CONTRIBUTIONS

BL contributed to Investigation, Validation, and Writing – original draft. WLL contributed to Software, Validation, and Writing – original draft. MLZ contributed to Investigation, Validation, and Software. YXW contributed to Software and Validation. YPD contributed to Funding acquisition, Supervision, and Writing – review & editing. XQM contributed to Software and Validation. JL contributed to Funding acquisition, Conceptualization, Supervision, Writing – original draft, and Writing – review & editing.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Young Star of Science and Technology Project of Dalian City (2021RQ007), Natural Science Foundation of Liaoning Province (2020-MS-246) and the Scientific Research Funding Project of the Liaoning Provincial Department of Education (LZ2020016).

ORCID ID

Jing Liu https://orcid.org/0000-0002-1468-7638

REFERENCES

1. Chen Y, Pu W, Maswikiti E, Tao P, Li X, Wang D, Gu B, Yu Y, Gao L, Zhao C, Chen H. Intestinal congestion and reperfusion injury: damage caused to the intestinal tract and distal organs. *Biosci Rep* 2021;**41**: BSR20211560
2. Yang J, Wu Y, Xu Y, Jia J, Xi W, Deng H, Tu W. Dexmedetomidine resists intestinal ischemia-reperfusion injury by inhibiting TLR4/MyD88/NF-κB signaling. *J Surg Res* 2021;**260**:350–8
3. Wang Z, Ji Y, Wang S, Wang R, Li Z, Kang A, Xu H, Shi M, Zhao M. Protective effect of intestinal ischemic preconditioning on ischemia reperfusion-caused lung injury in rats. *Inflammation* 2015;**38**:424–32
4. Li Y, Xu B, Xu M, Chen D, Xiong Y, Lian M, Sun Y, Tang Z, Wang L, Jiang C, Lin Y. 6-Gingerol protects intestinal barrier from ischemia/reperfusion-induced damage via inhibition of p38 MAPK to NF-κB signalling. *Pharmacol Res* 2017;**119**:137–48
5. Cho SS, Rudloff I, Berger PJ, Irwin MG, Nold MF, Cheng W, Nold-Petry CA. Remifentanil ameliorates intestinal ischemia-reperfusion injury. *BMC Gastroenterol* 2013;**13**:69
6. Li Y, Wen S, Yao X, Liu W, Shen J, Deng W, Tang J, Li C, Liu K. MicroRNA-378 protects against intestinal ischemia/reperfusion injury via a mechanism involving the inhibition of intestinal mucosal cell apoptosis. *Cell Death Dis* 2017;**8**:e3127
7. Goldsmith JR, Perez-Chanona E, Yadav PN, Whistler J, Roth B, Jobin C. Intestinal epithelial cell-derived μ-opioid signaling protects against ischemia reperfusion injury through PI3K signaling. *Am J Pathol* 2013; **182**:776–85
8. Wen J, Xu B, Sun Y, Lian M, Li Y, Lin Y, Chen D, Diao Y, Almoiliqy M, Wang L. Paeoniflorin protects against intestinal ischemia/reperfusion by activating LKB1/AMPK and promoting autophagy. *Pharmacol Res* 2019;**146**:104308
9. Doherty J, Baehrecke E. Life, death and autophagy. *Nat Cell Biol* 2018; **20**:1110–7
10. Murrow L, Debnath J. Autophagy as a stress-response and quality-control mechanism: implications for cell injury and human disease. *Annu Rev Pathol* 2013;**8**:105–37
11. He J, Liu J, Huang Y, Tang X, Xiao H, Hu Z. Oxidative stress, inflammation, and autophagy: potential targets of mesenchymal stem cells-based therapies in ischemic stroke. *Front Neurosci* 2021;**15**:641157
12. Mo Y, Sun YY, Liu KY. Autophagy and inflammation in ischemic stroke. *Neural Regen Res* 2020;**15**:1388–96
13. Rezq S, Hassan R, Mahmoud MF. Rimonabant ameliorates hepatic ischemia/reperfusion injury in rats: involvement of autophagy via modulating ERK- and PI3K/AKT-mTOR pathways. *Int Immunopharmacol* 2021;**100**:108140
14. Zhang Y, Liu D, Hu H, Zhang P, Xie R, Cui W. HIF-1α/BNIP3 signaling pathway-induced-autophagy plays protective role during myocardial ischemia-reperfusion injury. *Biomed Pharmacother* 2019;**120**:109464
15. Jing H, Luo F, Liu X, Tian X, Zhou Y. Fish oil alleviates liver injury induced by intestinal ischemia/reperfusion via AMPK/SIRT-1/autophagy pathway. *World J Gastroenterol* 2018;**24**:833–43
16. Wang A, Zhang H, Liang Z, Xu K, Qiu W, Tian Y, Guo H, Jia J, Xing E, Chen R, Xiang Z, Liu J. U0126 attenuates ischemia/reperfusion-induced apoptosis and autophagy in myocardium through MEK/ERK/EGR-1 pathway. *Eur J Pharmacol* 2016;**788**:280–5
17. Potenza MA, Sgarra L, Nacci C, Leo V, De Salvia MA, Montagnani M. Activation of AMPK/SIRT1 axis is required for adiponectin-mediated preconditioning on myocardial ischemia-reperfusion (I/R) injury in rats. *Plos One* 2019;**14**:e0210654
18. Ruderman NB, Xu XJ, Nelson L, Cacicedo JM, Saha AK, Lan F, Ido Y. AMPK and SIRT1: a long-standing partnership? *Am J Physiol Endocrinol Metab* 2010;**298**:E751–60
19. Carling D, Sanders MJ, Woods A. The regulation of AMP-activated protein kinase by upstream kinases. *Int J Obes (Lond)* 2008;**32**:S55–9
20. Li Z, Han X. Resveratrol alleviates early brain injury following subarachnoid hemorrhage: possible involvement of the AMPK/SIRT1/autophagy signaling pathway. *Biol Chem* 2018;**399**:1339–50
21. Zhu H, Ding J, Wu J, Liu T, Liang J, Tang Q, Jiao M. Resveratrol attenuates bone cancer pain through regulating the expression levels of ASIC3 and activating cell autophagy. *Acta Biochim Biophys Sin* 2017;**49**:1008–14
22. Guo S, Fu Y, Xiong S, Lv J. Corilagin protects the acute lung injury by ameliorating the apoptosis pathway. *Biomed Pharmacother* 2017;**95**: 1743–8
23. Ding Y, Ren D, Xu H, Liu W, Liu T, Li L, Li J, Li Y, Wen A. Antioxidant and pro-angiogenic effects of corilagin in rat cerebral ischemia via Nrf2 activation. *Oncotarget* 2017;**8**:114816–28
24. Jin F, Cheng D, Tao J, Zhang S, Pang R, Guo Y, Ye P, Dong J, Zhao L. Anti-inflammatory and anti-oxidative effects of corilagin in a rat model of acute cholestasis. *BMC Gastroenterol* 2013;**13**:79
25. Zhang R, Chu K, Zhao N, Wu J, Ma L, Zhu C, Chen X, Wei G, Liao M. Corilagin alleviates nonalcoholic fatty liver disease in high-fat diet-induced C57BL/6 mice by ameliorating oxidative stress and restoring autophagic flux. *Front Pharmacol* 2019;**10**:1693
26. Lv H, Hong L, Tian Y, Yin C, Zhu C, Feng H. Corilagin alleviates acetaminophen-induced hepatotoxicity via enhancing the AMPK/GSK3β-Nrf2 signaling pathway. *Cell Commun Signal* 2019;**17**:2
27. Souza DG, Pinho V, Soares AC, Shimizu T, Ishii S, Teixeira MM. Role of PAF receptors during intestinal ischemia and reperfusion injury. A comparative study between PAF receptor-deficient mice and PAF receptor antagonist treatment. *Br J Pharmacol* 2003;**139**:733–40
28. Wang G, Yao J, Li Z, Zu G, Feng D, Shan W, Li Y, Hu Y, Zhao Y, Tian X. miR-34a-5p inhibition alleviates intestinal ischemia/reperfusion-induced reactive oxygen species accumulation and apoptosis via activation of SIRT1 signaling. *Antioxid Redox Signal* 2016;**24**:961–73
29. Edlich F. BCL-2 proteins and apoptosis: recent insights and unknowns. *Biochem Bioph Res Co* 2018;**500**:26–34

30. Gupta A, Singh AK, Kumar R, Ganguly R, Rana HK, Pandey PK, Sethi G, Bishayee A, Pandey AK. Corilagin in cancer: a critical evaluation of anticancer activities and molecular mechanisms. *Molecules* 2019;**24**:3399
31. Pandey UB, Nie Z, Batlevi Y, McCray BA, Ritson GP, Nedelsky NB, Schwartz SL, DiProspero NA, Knight MA, Schuldiner O, Padmanabhan R, Hild M, Berry DL, Garza D, Hubbert CC, Yao TP, Baehrecke EH, Taylor JP. HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. *Nature* 2007;**447**:859–63
32. Li Y, Ye X, Zheng X, Chen W. Transcription factor EB (TFEB)-mediated autophagy protects against ethyl carbamate-induced cytotoxicity. *J Hazard Mater* 2019;**364**:281–92
33. Yang Y, Dong F, Liu X, Xu J, Wu X, Liu W, Zheng Y. Crosstalk of oxidative damage, apoptosis, and autophagy under endoplasmic reticulum (ER) stress involved in thifluzamide-induced liver damage in zebrafish (Danio rerio). *Environ Pollut* 2018;**243**:1904–11
34. Wu Y, Tang L, Wang B, Sun Q, Zhao P, Li W. The role of autophagy in maintaining intestinal mucosal barrier. *J Cell Physiol* 2019;**234**: 19406–19
35. Jin X, Chen M, Yi L, Chang H, Zhang T, Wang L, Ma W, Peng X, Zhou Y, Mi M. Delphinidin-3-glucoside protects human umbilical vein endothelial cells against oxidized low-density lipoprotein-induced injury by autophagy upregulation via the AMPK/SIRT1 signaling pathway. *Mol Nutr Food Res* 2014;**58**:1941–51
36. Kitada M, Ogura Y, Monno I, Koya D. Sirtuins and Type 2 diabetes: role in inflammation, oxidative stress, and mitochondrial function. *Front Endocrinol (Lausanne)* 2019;**10**:187
37. Tulino R, Benjamin A, Jolinon N, Smith D, Chini E, Carnemolla A, Bates G. SIRT1 activity is linked to its brain region-specific phosphorylation and is impaired in Huntington's disease mice. *Plos One* 2016;**11**:e0145425
38. Lee I, Cao L, Mostoslavsky R, Lombard D, Liu J, Bruns N, Tsokos M, Alt F, Finkel T. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci U S A* 2008;**105**:3374–9
39. Wei J, Wang Z, Zhong C, Ding H, Wang X, Lu S. LncRNA MIR503HG promotes hypertrophic scar progression via miR-143-3p-mediated Smad3 expression. *Wound Repair Regen* 2021;**29**:792–800
40. Wang X, Yu W, Sun Y. Activation of AMPK restored impaired autophagy and inhibited inflammation reaction by up-regulating SIRT1 in acute pancreatitis. *Life Sci* 2021;**277**:119435
41. Wang N, Luo Z, Jin M, Sheng W, Wang H, Long X, Wu Y, Hu P, Xu H, Zhang X. Exploration of age-related mitochondrial dysfunction and the anti-aging effects of resveratrol in zebrafish retina. *Aging (Albany NY)* 2019;**11**:3117–37
42. Li K, Liu T, Li J, Ma Y, Liu M, Wang Y, Wu R, Li B, Shi L, Chen C. rhEPO inhibited cell apoptosis to alleviate acute kidney injury in sepsis by AMPK/SIRT1 activated autophagy. *Biochem Biophys Res Commun* 2019;**517**:557–65

(Received September 25, 2022, Accepted November 15, 2022)