Ruscogenin Alleviates Intestinal Bleeding and Blood Flow Induced by Dasatinib through ROCK/MLC Pathway

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Research

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Abstract

Background

As a second-generation broad-spectrum tyrosine kinase inhibitor, Dasatinib has the antitumor effect of inhibiting tyrosine kinases such as BCR-ABL and SRC. It is mainly used to treat chronic and acute patients with chronic myelogenous leukemia. However, Dasatinib has many side effects, including gastrointestinal bleeding, respiratory infections, and renal failure, and effective means to improve the side effects of drugs are lacking.

Methods

C57BL/6 mice and HUVECs were used to evaluate the side effects caused by dasatinib, RUS was administered in advance to prevent, protein expression and phosphorylation were measured by western blot, and monitored the intestinal bleeding with Laser Doppler Blood Flow Monitor.

Results

We found that Ruscogenin (RUS) can improve Side effects caused by Dasatinib. Through the Laser Doppler Blood Flow Monitor, it was found that after Dasatinib was administered, blood flow in mice are decreased, and intestinal Evans blue leakage increased. Western blot results showed that connexin (ZO-1, VE-cadherin, Occludin) is destroyed, the activation of ROCK increases, and the phosphorylation of MLC increases. Ruscogenin can reverse the above phenomenon and improve the side effects of gastrointestinal bleeding caused by Dasatinib. I*n vitro*, giving Ruscogenin in advance can protect the endothelial barrier damage caused by Dasatinib, reduce the remodeling of F-actin, and restore the expression of connexin (ZO-1, VE-cadherin), inhibit the activation of ROCK, and reduce Phosphorylation of MLC.

Conclusions

This study provides a new direction for improving the side effects caused by clinical drugs and confirm that Ruscogenin could improve the side effect from Dasatinib by ROCK/MLC pathway.

Highlights

- **Intestinal bleeding and barrier destruction caused by gavage of Dasatinib in mice**
- **Ruscogenin protects the endothelial barrier function and reduces vascular leakage.**
- **Ruscogenin reverses the side effects of Dasatinib by inhibiting the ROCK/MLC signaling pathway**

1. Background

In clinical treatment, drug-induced side effects not only aggravate the patient's condition, but also bring a great financial burden to the patient[1]. A survey data shows that 71.3% of patients who require

hospitalization due to acute bleeding caused by drugs[2]. Therefore, prevention and treatment of side effects caused by drugs have a positive effect on the treatment of patients.

As a second-generation broad-spectrum tyrosine kinase inhibitor, Dasatinib exerts an anti-tumor effect by inhibiting tyrosine kinases such as BCR-ABL and SRC[3-4]. Clinically usemainly in the treatment of chronic myeloid leukemia and acute lymphoblastic leukemia[5], as well as leukemia patients resistant to Imatinib, Dasatinib inhibits wild-type BCR-ABL much stronger than the first-line treatment drug Imatinib At 325 times[6-7]. Dasatinib's global sales in 2016 were $1.8 billion. Although Dasatinib is very effective, it often shows serious side effects. The most common side effects in clinical reports include gastrointestinal symptoms[8], hemorrhagic events, hepatotoxicity[9], pleural effusion[10-12], pulmonary hypertension[13-14], and respiratory infections with neutral granules. Adverse events such as cell reduction and renal failure[15].

Clinical investigations shown that 23% of patients taking Dasatinib have developed bleeding, and gastrointestinal bleeding is a relatively high incidence of adverse reactions. Among patients with gastrointestinal bleeding, most patients received high doses of Dasatinib (> 100 mg) or received twice daily doses[16-18]. It can be judged that oral Dasatinib can cause gastrointestinal bleeding. The possible mechanism of Dasatinib's destruction of the endothelial barrier is due to the activation of ROCK / MLC signals, which causes actin polymerization and the breakdown of connexins in vascular endothelial cells. Intervention in the ROCK / MLC signaling pathway has been recognized for protecting the blood-brain barrier[19-20].Therefore, intervention of the endothelial ROCK-MLC signaling pathway and improvement of barrier disorders is to alleviate Dasatinib-induced intestinal bleeding.

At present, there are not many effective monitoring methods to examine the patient's bleeding.Some Articles have shown that an increase in organ bleeding can cause a decrease in blood flow[21-22].Most of the models used to study the side effects caused by Dasatinib are intraperitoneal injection or oral low-dose long-term administration[15,23-24]. Animal models of large-dose oral administration are lacking. In clinical use, large-dose oral administration is also common. Therefore, large-dose oral administration was selected.

Ruscogenin is one of the effective active ingredients of *Ophiopogon japonicus*, which has effect on anti-inflammatory, anti-thrombosis and reduces capillary permeability[25-26]. Ruscogenin can alleviate endothelial cell injury, protect blood-brain barrier function, and relieve pulmonary hypertension caused by monocrotaline[27-28].In the early stage of the laboratory, It was proved that Ruscogenin can up-regulate the expression of adhesion proteins VE-cadherin and p120-catenin, inhibit the nuclear translocation of VE-cadherin, thereby improving the adhesion of endothelial cell adhesion junction and reducing the damage of the pulmonary vascular endothelium induce by Lipopolysaccharide[29] .

Based on the above data, we hypothesized that RUS can be used as a clinical intervention to improve the side effects of Dasatinib causing endothelial dysfunction.

2. Materials And Methods

2.1 Materials

Ruscogenin, 98%, from J&K® (Beijing China).Flux of Evans blue dye-labeled albumin (EB-albumin, Sigma, USA).

2.2 Animals

All animal care and experimental procedures were carried out according to the current European Communities Council-ECC guidelines for the care of laboratory animals and ethical guidelines for investigations of experimental pain in conscious animals. Animal studies were reported in compliance with the ARRIVE guidelines. Male C57BL/6J mice (20–25 g; 8 weeks; specified pathogen free) were provided by the Model Animal Research Centre of Yangzhou University (Yangzhou, Jiangsu, China) and kept in cages containing standard bedding, with at least five mice per cage. Mice were housed in a specific pathogen-free facility with 12 h light/dark cycle [07 to 19 h, temperature (22 ± 2°C), humidity (40–70%), controlled ventilation] and with sterile water and irradiated food available *ad libitum*. Animals were allowed to acclimatize to their housing environment for at least 7 days prior to experimentation and to the experimental room for 1 h before experiments.

2.3 Drug treatments

Ruscogenin was weighed and dissolved in absolute ethanol to prepare a mother liquor. When used, the corresponding concentration was adjusted with physiological saline. Dasatinib was dissolved in physiological saline at a concentration of 140 mg/kg and administered intragastrically.30 minutes after the administration of Ruscogenin, Dasatinib was administered by gavage.After 30 minutes, the mice were killed.

2.4 Blood flow monitoring

Thirty minutes before administering Dasatinib, mice were given Ruscogenin, and 2 hours later anesthetized with Pelltobarbitalum Natricum. The small intestine was removed and placed on a Doppler flowmeter for scanning to obtain images.

2.5 Western blot analysis

Western blotting analysis was performed as previously described. The cells was lysed and centrifuged. Equal amounts of proteins (30 μg) were examined using 12.5% SDS-PAGE and subsequently transferred to PVDF membranes (Millipore, USA) using electrophoresis. The membrane was blocked with 5% BSA for 2 h and incubated overnight at 4 °C with primary antibodies against ZO-1 (1:200,Abcam,USA), VE-cadherin（1:1000，Santa，USA） Occludin(1:200,Abcam,USA)， ROCK1 (1:500, Santa, USA), MLC (1:1000, CST, USA), phospho-MLC (1:1000, CST, USA),β-actin (1:10000，Bioworld，USA), followed byincubation with peroxidase-conjugated secondary antibodies (1:8000, Bioworld, Louis Park, USA) and visualization using

enhanced chemiluminescence (ECL,Vazyme Biotech, Nanjing, China). The results were quantified using the Image Lab™ software (version 4.1, Bio-Rad, California, USA).

2.6 Histology and immunohistochemistry

The excised vessel was dehydrated with 40% sucrose, embedded in optimal cutting temperature (OCT) and frozen at −70°C. The jejunum was sectioned into slices of 10 μm of thickness with a cryotome (Leica, Mannheim, Germany). Specimens were washed in PBS and stained with haematoxylin and eosin (H&E). For immunohistochemical staining, slides were incubated with primary antibodies at 4°C overnight. Alexa Fluor 488-/594-labelled antibodies were used as secondary antibodies. The nuclei were stained with DAPI(Beyotime Biotechnology, Shanghai, China). Pathological changes in jejunumwere observed under a fluorescence microscope (Leica, Mannheim, Germany).

2.7 Cell culture

HUVECs were purchased from the Bioleaf Biotech Co. Ltd. (Shanghai, China) and cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO, USA), 100 U/ml penicillin and 100U/ml streptomycin (Ameresco, OH, USA) at 37 °C in a humidified atmosphere of 5% CO2 and 95% air.

2.8Dasatinib-induced disruption of HUVECs barrier

In the control group, serum-free RPMI-1640 medium was added to the chamber for 2 h. Dasatinib (final concentration 150 nM) was added to the model group for 2 h. The administration group was added with 0.01, 0.1, 1 μM RUS for 1 h, and then 150 nM Dasatinib was added for 2 h.

2.9 Statistical analysis.

All results are expressed as the means±SD. Statistical analysis was performed using Student’s two-tailed t test for comparison between two groups and one-way analysis of variance (ANOVA) followed by Dunnett’s test when the data involved three or more groups. $P<0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism Version 5.01 (GraphPad Software Inc.USA).

3. Results

3.1 Oral Dasatinib reduced intestinal blood flow in mice

Clinically, Dasatinib is used for oral treatment of chronic myeloid leukemia. In this section, the time points of blood flow reduction in the small intestine caused by Dasatinib were observed by different doses of Dasatinib (70, 140, 210 mg/kg). As shown in Fig.1, administration of 70 mg/kg Dasatinib did not caused evident decrease blood flow in intestinal . Compared with the control group, the blood flow in small intestine was significantly decreased ($P<0.01$) when the gavage of 140 mg/kg Dasatinib was

administered for 90 minutes, and the blood flow of the small intestine continued to decrease at 135 min (P<0.01). When the mice were given by *gavage* 210 mg/kg Dasatinib for 45 min, the blood flow in the small intestine was significantly decreased (P<0.01), and the blood flow in the small intestine was continuously decreased at 90 and 135 min (P<0.01).

3.2 RUS reverted the Intestinal barrier leakage in C57BL/6 subjected to Dasatinib

We found that oral Dasatinib caused a decrease in intestinal blood flow. Therefore, we used the Evans blue method to detect the effects of Dasatinib-induced small intestinal vascular leakage in mice. The results are shown in Fig. 2A. Compared with the control group, the leakage rate of Evans blue in the small intestine of the model group was significantly increased (P<0.001), and the inhibitory effect of RUS at the high dose was the best (P<0.001). This indicates that RUS has an obvious effect on intestinal vascular leakage caused by Dasatinib As shown in Fig. 2C, 2D ,RUS at 30μg/kg reversed the blood flow reduction caused by Dasatinib at 90min(P<0.05). HE staining showed that RUS alleviated Dasatinib-induced intestinal barrier destruction and hemorrhage.

3.3 RUS ameliorated the expression of junction and adhesion proteins in C57BL/6subjected to Dasatinib

We investigated the effect of RUS on connexin expression by Western blot. The results of protein imprinting showed that compared with the control group, the expression of junction proteins ZO-1,Occludin and VEcadherin in the model group were significantly decreased (P<0.001 & P<0.05), and RUS at high dose significantly inhibited Dasatinib-induced ZO-1 and Occludin. VEcadherin expression decreased (P<0.001 & P<0.01).Immunofluorescence results showed that RUS increased the expression of ZO-1.

3.4 RUS ameliorated the side effects of Dasatinib by inhibiting the ROCK1 / MLC signaling pathway

Hemorrhagic complications of Dasatinib mainly phosphorylate MLC through the ROCK pathway, resulting in cytoskeletal remodeling; therefore, we investigated whether RUS can reduce the phosphorylation of MLC by inhibiting the activation of ROCK-1, thereby protecting the intestinal barrier. Western blot results showed that high dose RUS significantly inhibited Dasatinib-induced ROCK-1 activation and phosphorylation of MLC(P<0.001 & P<0.05).

3.5 RUS reverted the barrier leakage in HUVECs subjected to Dasatinib.

As the Fig.5 shows that the HUVECs were treated with RUS (0.01-1μM), subsequently exposed to 2h of Dasatinib (150 nM), and the barrier protective effect of RUS was evaluated using EB albumin assays in HUVECs.The results demonstrated that treatment with RUS can ameliorates endothelial barrier function after 2 h of Dasatinib(P<0.01).We also observed the effect of RUS on the changes in F-actin using an immunofluorescence assay. The results demonstrated that RUS stabilized the cytoskeleton and reduced the incidence of cell contraction leading to cell shrinkage in HUVECs subjected to Dasatinib .

3.6 RUS improved the expression of junction and adhesion proteins in HUVECs cells subjected to Dasatinib

First, we investigated the effects of RUS on the expression of junction and adhesion proteins after 2 h of Dasatinib. The results of the western blot analysis indicated that treatment with RUS(0.1μM&1μM) up-regulated the decreased expression of ZO-1 and VEcadherin after 2 h of Dasatinib in HUVECs . These findings suggested that RUS improved the Dasatinib-induced abnormal expression of junction and adhesion proteins in HUVECs(P<0.001 & P<0.01).

Considering the role of the ROCK/MLC pathway in microvascular endothelial barrier function, we also used western blotting to evaluate the effect of RUS on the expression of proteins associated with the ROCK/MLC pathway. The results showed that treatment with RUS (0.1μM and 1μM) inhibited ROCK1 inhibited, and reduced MLC phosphorylation (P<0.01 & P<0.05). These results indicated that the ROCK/MLC pathway was also involved in the RUS-induced improvement in microvascular endothelial barrier function.

4. Discussion

The side effects of clinical drugs have always been a major problem for patients' rehabilitation. Drugs that cause serious adverse reactions, even included some commonly used clinical drugs, such as oral antiplatelet drugs, warfarin, oral hypoglycemic drugs, etc[2].Common adverse reactions include bleeding events, hypoglycemia, arrhythmia, and so on，lack effective preventive intervention and monitor methods[30].Therefore, it is of great significance to find and develop bioactive molecules that can prevent adverse drug reactions.

In order to find a way to ameliorate the hemorrhagic side effects induced by Dasatinib in clinical practice, we selected Ruscogenin, an active ingredient derived from the natural drug, *Ophiopogon japonicus*. Experiments have shown that RUS can significantly inhibit Dasatinib-induced blood flow decreased at intestinal, through by Evans blue shown that RUS can improved intestinal vascular leakage, and up-regulated the expression of connexin ZO-1, Occludin, VEcadherin, and inhibited ROCK activation and MLC phosphorylation.RUS Inhibit the remodeling of the endothelium of blood vessels, exerting the protective effect of the endothelium and inhibiting the adverse reactions caused by Dasatinib.

In order to simulate the clinical use of Dasatinib better[31], we observed it by high-dose oral administration Dasatinib and found that RUS can display a certain protective effect.In patients with intracerebral hemorrhage, monitoring found that the patient's cerebral blood flow decreased[22], so we suspected that monitoring intestinal blood flow can reflect intestinal bleeding. The experimental results showed that after administration of Dasatinib by gavage, the intestinal blood flow of mice significantly decreased (see Fig. 1).Through this way,we provide a monitoring method for patients who need long-term to take Dasatinib or similar drugs that can cause adverse events like bleeding. After oral administration of Dasatinib, the small intestine structure of the mice was destroyed, bleeding increased, and vascular

leakage increased. Pre-administration of RUS reversed the decrease in blood flow and reduced leakage of Evans Blue(see Fig. 2).

Only recently, the intestinal vascular endothelium is recognized as an important barrier in the intestine[32-33].Vascular endothelial cell is one of the main components of intestinal mucosa.Protecting the intestinal endothelial barrier and maintain vascular stability is become a new way to treat intestinal diseases[34-36]. The destruction of connexin will affect the integrity of intestinal blood vessels, increase the permeability of blood vessels, and cause leakage of blood vessels. Therefore, connexin plays a significant role in stabilizing intestinal permeability.Tight junctions are essential for regulating intestinal permeability[37-38]. The tight junction proteins in EC include ZO-1, claudin-5, occludin and several JAMs. Previous studies have shown that ZO-1, occludin protein could be regarded as a characteristic marker of TJ, and down-regulation of these proteins lead to undermining the tight junctions[39]. Among them, ZO-1 is a junctional adaptor protein that interacts with multiple other junctional components.It can regulate the formation of blood vessels in vitro and in vivo, maintain the connection tension, and at the same time, ZO-1 binds to F-actin and is involved in the regulation of cytoskeleton by actomyosin[40].The endothelial cytoskeleton plays an important role in maintaining the structure of endothelial cells.VE-cadherin is a protein specifically expressed in endothelial cells and plays an important role in maintaining adhesion junctions. The steady-state dynamics of VE-cadherin at AJs is a critical determinant of AJ integrity[41].There is a close relationship between VE-cadherin and ZO-1[40]. A recent study shows that protecting the endothelial barrier has become a new strategy for treating inflammatory bowel disease[42-43].All in all, adherens junctions and tight junctions play an important role in maintaining the endothelial barrier. Early administration of RUS can protect the expression of connexin. Compared with the model group, the expressions of ZO-1, VE-cadherin, and Occludin are all increased, and the results are the same in HUVECs in vitro(see Fig. 3 and Fig.6).

The cytoskeletal protein F-actin polymerization produces a mechanical force to change the cell morphology. Therefore, the integrity of the endothelial cytoskeletal protein F-actin structure is the basic structural for maintaining the barrier function of endothelial cells. Dasatinib phosphorylates MLC via the ROCK pathway[24,44],induces the polymerization of F-actin structure, remodels the cytoskeleton, and destroys the barrier function of endothelial cells. In view of the function of RUS to protect endothelial cells, we speculate that RUS can inhibit ROCK activation, reduce MLC phosphorylation, reduce cytoskeleton remodeling, restore connexin expression, and play a protective role by interfering with the ROCK-MLC signaling pathway(see Fig.4 and Fig.6).

Dasatinib has excellent efficacy in the clinical treatment of leukemia, but as a protein kinase inhibitor, it is bound to affect the function of the endothelial barrier. It is well known that many different phosphatases and kinases can act as regulators of endothelial barrier function[45-47]. After taking Dasatinib, it destroys the original regulatory function, causing damage to the endothelium, which causes numerous adverse reactions. Therefore, before we use these drugs with a high risk of side effects , we can match to some

drugs with hemostasis, barrier protection and regulation related signaling pathways to bring more benefits to patients.Some natural products have good development potential[48].

Ruscogenin is derived from traditional Chinese medicine, *Ophiopogon japonicus*,it can anti-inflammatory [49], anti-thrombosis and protective barrier function[19]. It also can prevent the occurrence of hemorrhagic events.Due to the lack of effective interventions for the bleeding side effects caused by Dasatinib, we considered that Ruscogenin has the function of protecting the endothelial barrier, and there are no clear side effects reported, so we assumed that Ruscogenin can improve the side effects caused by Dasatinib through protects barrier.The use of traditional Chinese medicine to regulate the side effects of other drugs has been initially recognized as a new treatment strategy[50].

5. Conclusion

In summary, we provided a new animal model for studying the side effects of Dasatinib, and provided a method for clinical monitoring of gastrointestinal bleeding in patients, and found that a natural product can alleviate the side effects caused by Dasatinib. Whether RUS is effective for long-term use of Dasatinib or gastrointestinal bleeding caused by Dasatinib in pathological conditions, and whether RUS can protect the vascular endothelial barrier through other signaling pathways remains to be explored[51-53]. This study broadens the clinical application of RUS in the future, provides pharmacological evidence for RUS prevention and treatment of hemorrhagic side effects, and provides clues for the subsequent development of natural drugs.

Abbreviations

BSA(Bovine serum albumin); ;ECL(Enhanced chemiluminescence)；FBS(Fetal bovine serum); HRP(Horse radish peroxidase); HUVECs(Human umbilical vein endothelial cells); MLC(Myosin Light Chain); ROCK(Rho-associated kinase); PVDF(Polyvinylidene fluoride); SDS(Sodium dodecylsulfate); AJs ：Adherens junction；TJs(Tight junctions).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors and Contributors

Yuanyuan Zhang, Junping Kou and Boyang Yu designed experiments. Yuankai Liu Yujie Dai and Han Xu performed experiments.Yuankai Liu wrote the manuscript. Yuanyuan Zhang and Junping Kou polished the language. All authors contributed toward editing the manuscript.

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Figures

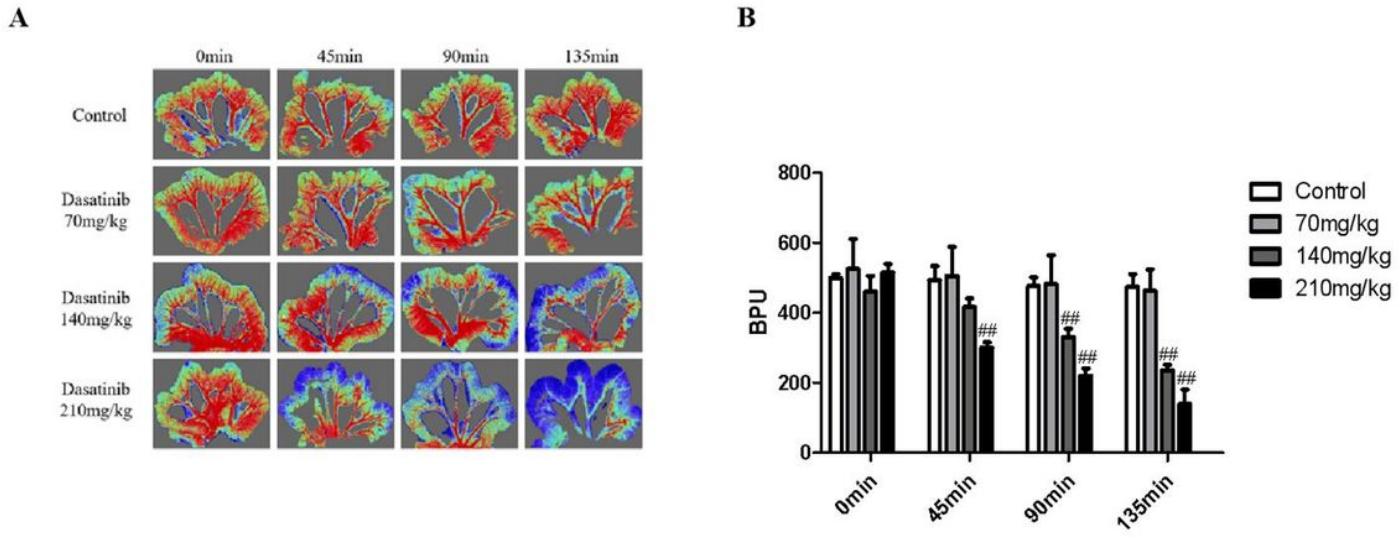

Figure 1

The change of intestinal blood flow in mice with Dasatinib .Mice were orally administered with Dasatinib (70、140、210 mg/kg). The representative images of intestinal blood flow in different groups. The magnitude of IBF is represented by different colors, with blue to red denoting low to high,Quantitative analysis of IBF in different groups. Data are expressed as the mean±SD, n=6.#P<0.05 vs.Control,##P<0.01 vs.Control,###P<0.001 vs.Control; *P<0.05 vs. Model, **P<0.01 vs.Model,***P<0.001 vs.Model.

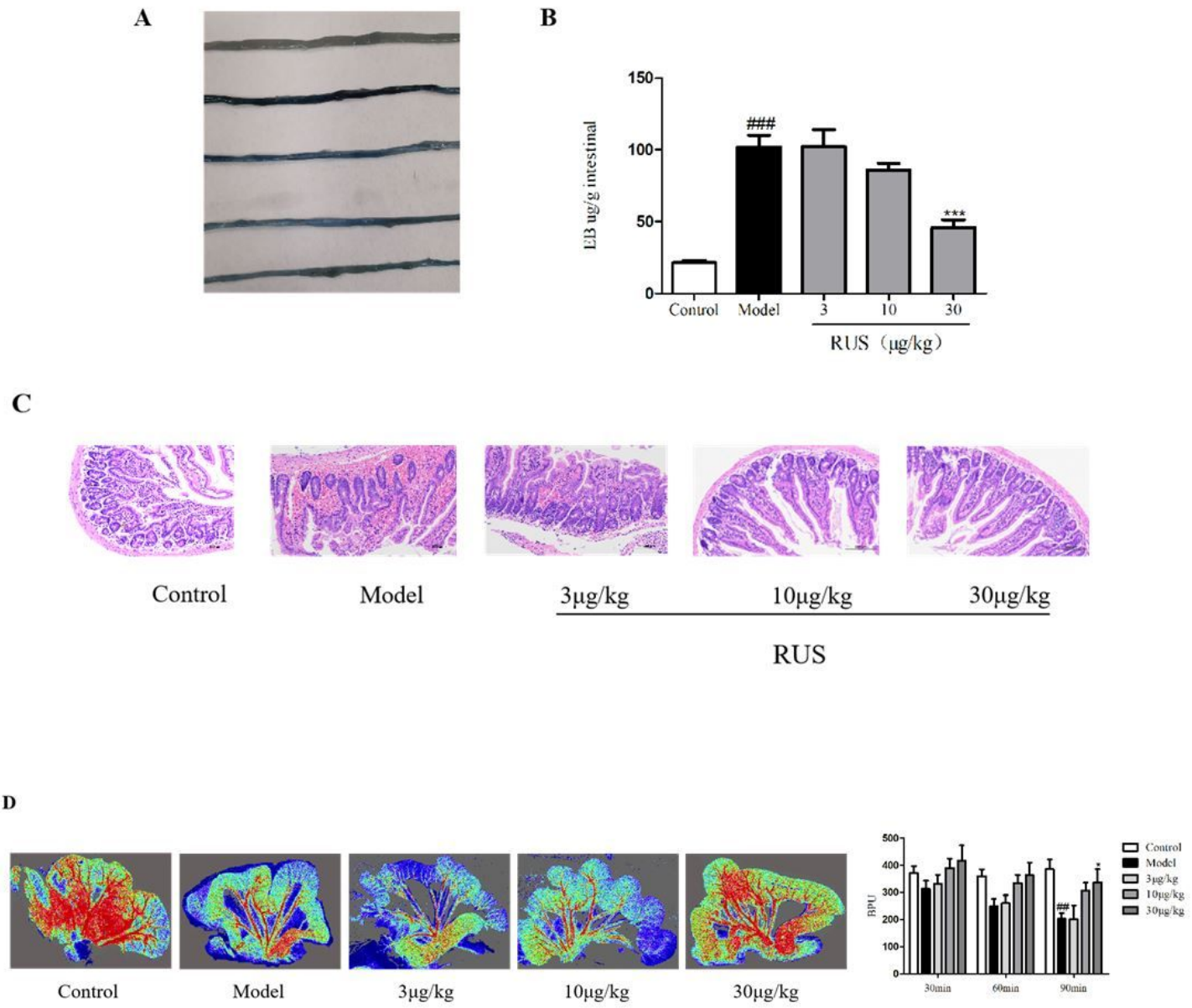

Figure 2

Protective effect of RUS on side effects caused by Dasatinib.Mice were orally administered with RUS (3、10、30 μg/kg) prior to Dasatinib (140 mg/kg,90 min, po) .A-B) Representative gross appearance of EB-stained intestinal from mouse. Quantitative analysis of EB extravasation by spectrophotometry.Data are expressed as the mean ± SD, n=6. ##P<0.01vs. Control mice; **P<0.01 vs. Model mice. C) Hematoxylin-eosin stained slides of mouse intestinal sections in different groups were examined under a light microscope, and representative stained sections showed RUS improves Dasatinib-induced bleeding. Scale bar =100 μm. n=3.D) The representative images of intestinal blood flow in different groups. The magnitude of IBF is represented by different colors, with blue to red denoting low to high. Quantitative analysis of IBF in different groups. Data are expressed as the mean±SD, n=6. #P<0.05 vs.Control,##P<0.01 vs.Control,###P<0.001 vs.Control; *P<0.05 vs. Model, **P<0.01 vs.Model,***P<0.001 vs.Model.

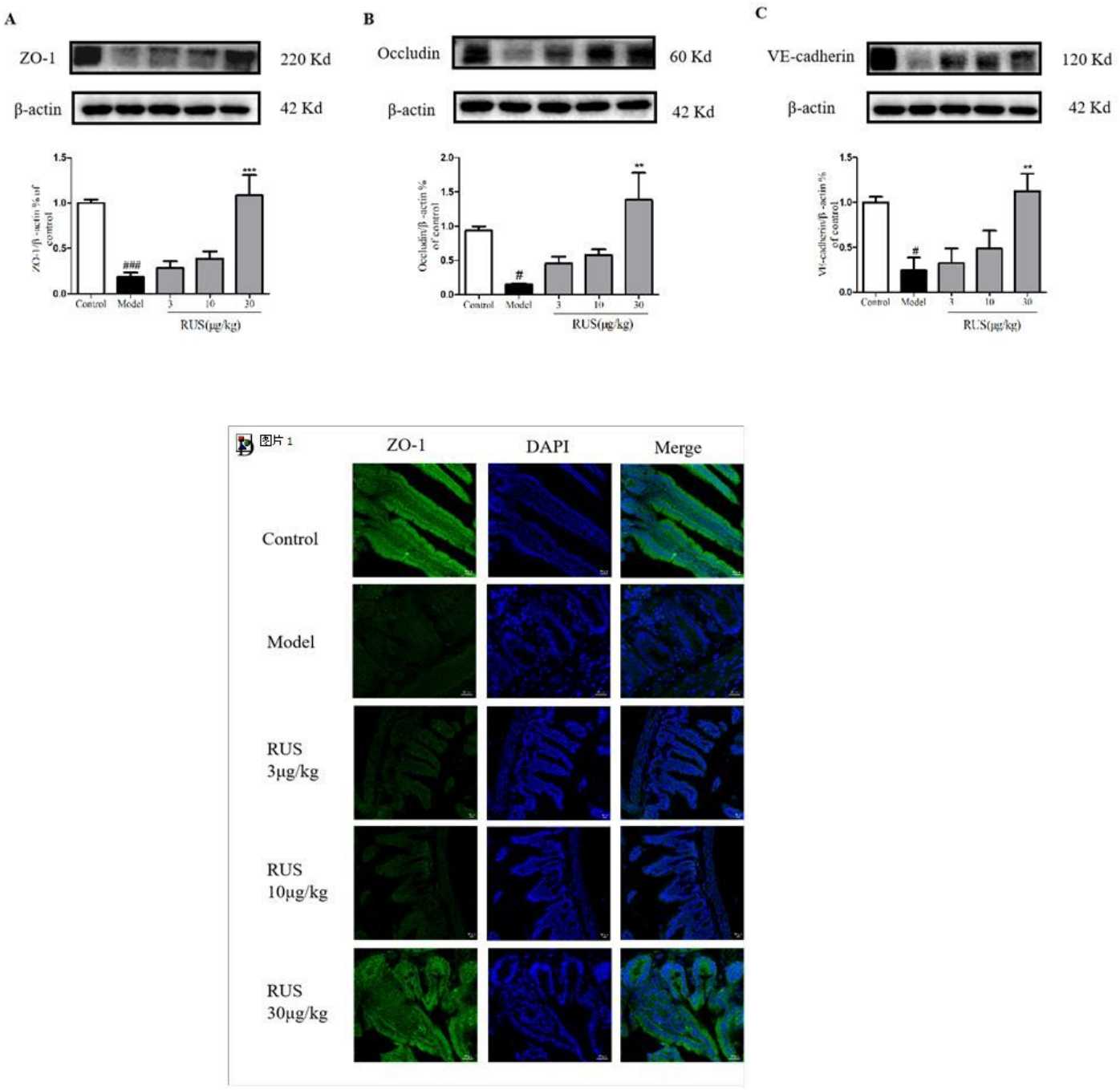

Figure 3

Effect of RUS on the expression of junction proteins in mice with Dasatinib. Mice were orally administered with RUS (3,10,30μg/kg) prior to Dasatinib (140 mg/kg, po). A-C) Representative Western blots and the quantitative analysis of the ratio of ZO-1,Occludin,VE-cadherin. Data are expressed as the mean±SD, n=4. #P<0.05 vs.Control,##P<0.01 vs.Control,###P<0.001 vs.Control; *P<0.05 vs. Model, **P<0.01 vs.Model,***P<0.001 vs.Model. D) Representative confocal microscopy images showing the immunostaining of ZO-1 in mice.Scale bar =100 μm. n=3.

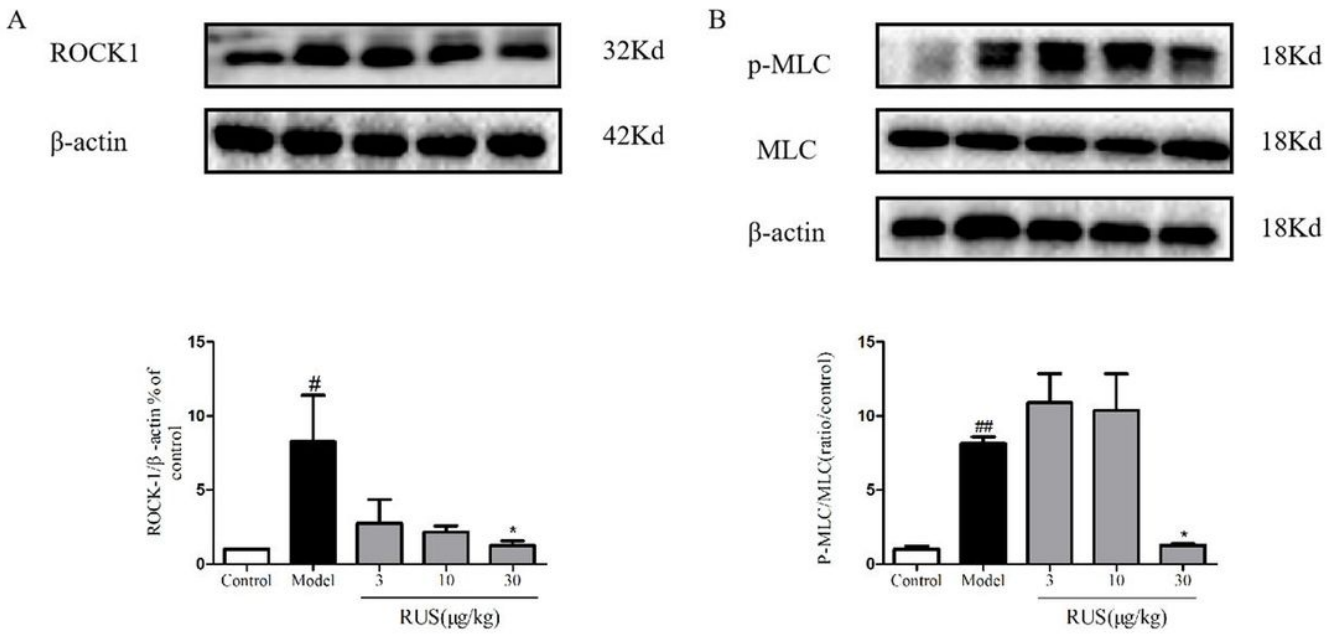

Figure 4

Effect of RUS on the ROCK1/MLC signaling pathway in mice with Dasatinib. Mice were orally administered with RUS (3,10,30μg/kg) prior to Dasatinib (140 mg/kg, po). A) Western blots and the quantitative analysis of the ratio of ROCK-1, B) Western blots and the quantitative analysis of the ratio of P-MLC. Data are expressed as the mean±SD, n=4. #P<0.05 vs.Control,##P<0.01 vs.Control,###P<0.001 vs.Control; *P<0.05 vs. Model, **P<0.01 vs.Model,***P<0.001 vs.Model.

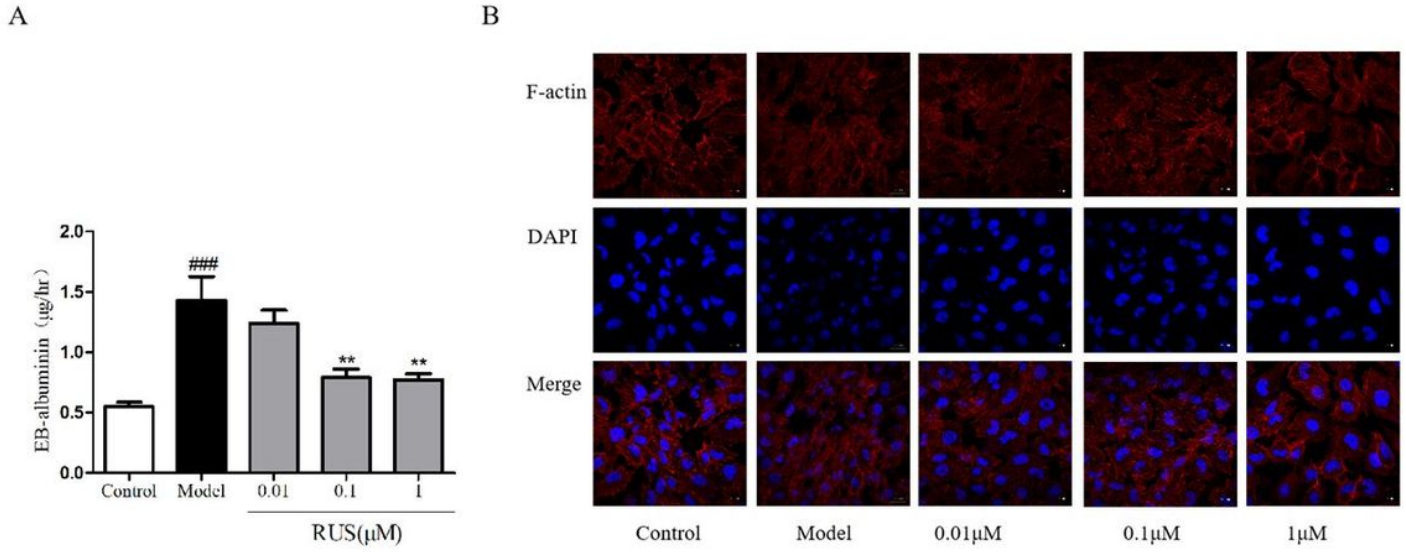

Figure 5

Effects of RUS on barrier function in HUVECs subjected to Dasatinib.The HUVECs were treated with RUS (0.1-1 μM) 1h, exposed to 2 h of Dasatinib. (A, B)The barrier-protective effect of RUS was evaluated using EB albumin assays and Representative confocal microscopy images showing the immunostaining of F-actin in HUVECs. The Data are expressed as the mean±SD, n=3. #P<0.05 vs.Control,##P<0.01 vs.Control,###P<0.001 vs.Control; *P<0.05 vs. Model, **P<0.01 vs.Model,***P<0.001 vs.Model.

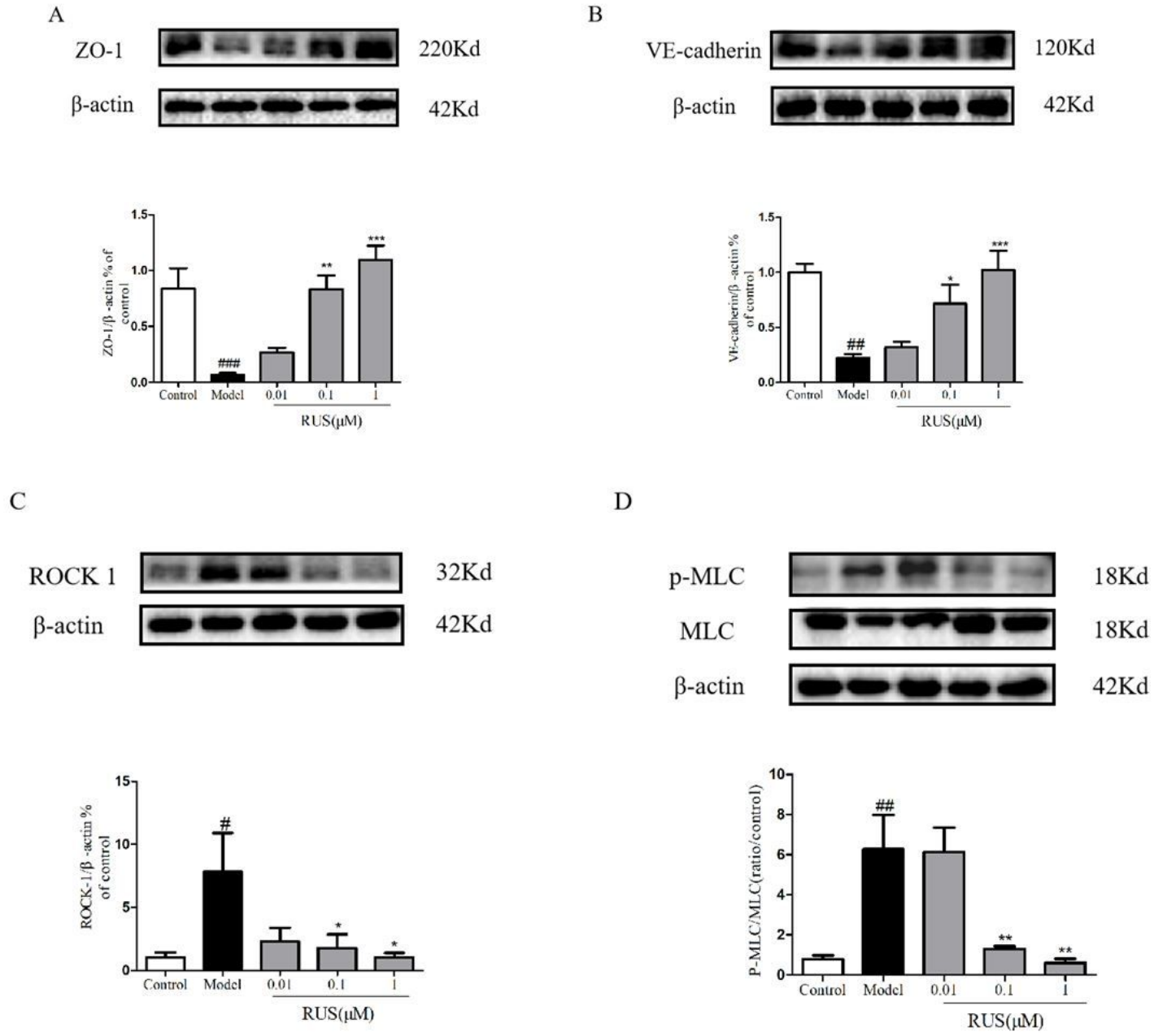

Figure 6

Effects of RUS on the expression of junction proteins and ROCK1/MLC signaling pathway in HUVECs subjected to Dasatinib.(A,B) The HUVECs were treated with RUS(0.01-1μM)1h, followed by 2 h of Dasatinib. The expression levels of ZO-1, VE-cadherin levels were measured using Western blotting. The band intensities were assessed using scanning densitometry, and the data were normalized against the β-actin signal. The Data are expressed as the mean±SD, n=4. #P<0.05 vs.Control,##P<0.01 vs.Control,###P<0.001 vs.Control; *P<0.05 vs. Model, **P<0.01 vs.Model,***P<0.001 vs.Model. (C,D) The

HUVECs were treated with RUS(0.01-1μM)1h, followed by 2 h of Dasatinib. The expression levels of ROCK-1,P-MLC levels were measured using Western blotting. The expression levels of ROCK1, MLC and p-MLC were determined using Western blotting analysis.The band intensities were assessed through scanning densitometry. The Data are expressed as the mean±SD, n=4.#P<0.05 vs.Control,##P<0.01 vs.Control,###P<0.001 vs.Control; *P<0.05 vs. Model, **P<0.01 vs.Model,***P<0.001 vs.Model.

Supplementary Files

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