Research Article

Protective Effects of Cinnamaldehyde against Mesenteric Ischemia-Reperfusion-Induced Lung and Liver Injuries in Rats

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The aim of this study was to characterize and reveal the protective effects of cinnamaldehyde (CA) against mesenteric ischemia-reperfusion- (I/R-) induced lung and liver injuries and the related mechanisms. Sprague-Dawley (SPD) rats were pretreated for three days with 10 or 40 mg/kg/d, ig of CA, and then induced with mesenteric ischemia for 1 h and reperfusion for 2 h. The results indicated that pretreatment with 10 or 40 mg/kg of CA attenuated morphological damage in both lung and liver tissues of mesenteric I/R-injured rats. CA pretreatment significantly restored the levels of aspartate transaminase (AST) and alanine transaminase (ALT) in mesenteric I/R-injured liver tissues, indicating the improvement of hepatic function. CA also significantly attenuated the inflammation via reducing myeloperoxidase (MOP) activity and downregulating the expression of inflammation-related proteins, including interleukin-6 (IL-6), interleukin-1β (IL-1β), cyclooxygenase-2 (Cox-2), and tumor necrosis factor receptor type-2 (TNFR-2) in both lung and liver tissues of mesenteric I/R-injured rats. Pretreatment with CA significantly restored the levels of aspartate transaminase (AST) and alanine transaminase (ALT) in mesenteric I/R-injured liver tissues, indicating the improvement of hepatic function. CA also significantly attenuated the inflammation via reducing myeloperoxidase (MOP) activity and downregulating the expression of inflammation-related proteins, including interleukin-6 (IL-6), interleukin-1β (IL-1β), cyclooxygenase-2 (Cox-2), and tumor necrosis factor receptor type-2 (TNFR-2) in both lung and liver tissues of mesenteric I/R-injured rats. CA pretreatment with CA significantly downregulated nuclear factor kappa B (NF-κB) related protein expressions (NF-κB p65, NF-κB p50, I kappa B alpha (Ik-α), and inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ)) in both lung and liver tissues of mesenteric I/R-injured rats. CA pretreatment with CA significantly downregulated nuclear factor kappa B (NF-κB) related protein expressions (NF-κB p65, NF-κB p50, I kappa B alpha (Ik-α), and inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ)) in both lung and liver tissues of mesenteric I/R-injured rats. CA also significantly downregulated the protein expression of p53 family members, including caspase-3, caspase-9, Bax, and p53, and restored Bcl-2 in both lung and liver tissues of mesenteric I/R-injured rats. CA pretreatment with CA significantly reduced TUNEL-apoptotic cells and significantly inhibited p53 and NF-κB p65 nuclear translocation in both lung and liver tissues of mesenteric I/R-injured rats. CA neither induced pulmonary and hepatic histological alterations nor affected the parameters of inflammation and apoptosis in sham rats. We conclude that CA alleviated mesenteric I/R-induced pulmonary and hepatic injuries via attenuating apoptosis and inflammation through inhibition of NF-κB and p53 pathways in rats, suggesting the potential role of CA in remote organ ischemic injury protection.
1. Introduction

Mesenteric I/R injury is a serious pathological condition with characteristics of hemorrhagic shock, trauma, strangulated intestinal obstruction, and acute mesenteric ischemia (AMI) [1–3]. Mesenteric I/R injury may induce remote organ injuries, including lung and liver injuries which are associated with high morbidity and mortality [4–6]. Mesenteric I/R-induced pulmonary injury may lead to either acute dysfunction or severe dysfunction (failure), which may further cause myocardial, hepatic, and renal failure followed by death [7–9]. Mesenteric I/R injury is a clinical challenge, and the effective therapeutic strategy is limited with the exception of surgery [10–12], showing the requirement of novel treatment options for ameliorating both direct mesenteric I/R injury and indirect remote organ injuries.

Protective effects induced by natural compounds are found to ameliorate mesenteric I/R-induced local and/or remote organ injuries. For instance, ginsenoside Rb1 ameliorates mesenteric I/R-induced lung injury [13]; curcumin alleviates pulmonary and renal injuries induced by mesenteric I/R, respectively [14, 15]; and ghrelin ameliorates mesenteric I/R-induced liver injury [16]. Irisin protects against mesenteric I/R-induced liver injury [17]. And in our previous study, CA attenuates mesenteric I/R-induced gut injury via a synergistic inhibition of p53/NF-κB signaling pathways [18].

Cinnamaldehyde (CA, Figure 1) is the active constituent of cinnamon extract obtained from the bark of Cinnamomum [19, 20]. CA has various beneficial effects, such as anti-bacterial [21], anti-inflammatory [22], antioxidative [23], and antiapoptotic effects [24]. CA is found to protect against gram-positive/negative infection [25], diabetes [26], gastric ulcer [27], cardiac hypertrophy [28], and myocardial [29]/brain I/R injuries [30, 31]. However, whether CA can efficiently protect against mesenteric I/R-induced injuries in the lung and liver still needs to be revealed. Based on our pre-experiments, we proposed that CA pretreatment could ameliorate mesenteric I/R-induced liver and lung injuries via attenuating inflammation and apoptosis through inhibition of both NF-κB and p53 signaling pathways. Rat models of mesenteric I/R-induced lung and liver injuries were used to verify our proposal.

2. Materials and Methods

2.1. Chemicals and Materials. From Aladdin (Aladdin, Shanghai, China), cinnamaldehyde (purity: ≥98%) was purchased. The assay kits for detecting alanine transaminase (ALT), aspartate transaminase (AST), and myeloperoxidase (MPO) and an assay of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) were obtained from Nanjing Jiancheng Institute of Biotechnology (Nanjing, Jiangsu, China). The protein extraction kits, bicinchoninic acid protein assay kits, and hematoxylin and eosin staining kits were obtained from Beyotime Institute of Biotechnology (Haimen, Jiangsu, China). 4′,6-Diamidino-2-phenylindole (DAPI) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Animals. 200-220 g male Sprague-Dawley (SPD) rats were provided by the Animal Center (Dalian Medical University) (certificate of conformity: NO.SCXK (Liao) 2018-0003). According to the National Institutes of Health guideline (publication no. 85-23, revised 1985) and Dalian Medical University (approval number: L20140402) protocols, rats were received care and housed one per cage under daily hygiene and proper environment.

2.3. Induction of Mesenteric I/R Model. Mesenteric I/R injury on a rat model was induced as previously described [32, 33]. 12 h before the I/R injury, the animals were fasted of food, and then, on the day of the experiment, the rats were anesthetized with pentobarbital (50 mg/kg of body weight) intraperitoneally (ip). The superior mesenteric artery (SMA) was clamped with a traumatic microvascular clamp for 1 h to achieve ischemia; then, the SMA was unclamped for an additional 2 h to induce reperfusion. And the lung and liver tissue samples were collected and placed on ice, rinsed with phosphate-buffered saline (PBS), and stored at -80°C after the rats were euthanized. Segments of lung and liver tissue were fixed with formalin for TUNEL, immunofluorescence, and histological analysis.

The rats were randomly assigned to 5 groups (5 rats/group): (1) sham group: via the intragastric gavage (ig) route, rats were given a vehicle daily for 3 days before rat sham surgery; (2) sham+CA group: rats were subjected to sham surgery after pretreatment with CA (ig) with the concentration 40 mg/kg daily/3 days; (3) I/R group: via the intragastric gavage (ig) route, rats were given a vehicle daily for 3 days before they were induced with 1 h mesenteric ischemia and then reperfusion for additional 2 h; (4) I/R+CA (L) group: rats were subjected to I/R surgery after they were pretreated with CA (ig) with the concentration 10 mg/kg/day/3 days [18, 34]; and (5) I/R+CA (H) group: rats were subjected to I/R surgery after they were pretreated with CA (ig) with the concentration 40 mg/kg/day/3 days [18, 35, 36]. The intragastric gavage suspension of CA in carboxymethyl cellulose (1% CMC) was prepared daily and freshly and given at 2 mL/kg.

2.4. Tissue Staining and Histology. Hematoxylin and eosin (H&E) (H&E staining®, Haimen, Jiangsu, China) staining was performed after the lung and liver tissue samples were fixed in formalin solution, paraffinized, and then sliced. The samples were randomly selected and stained with H&E staining according to the manufacturer’s instruction (H&E staining®, Haimen, Jiangsu, China). The extent of I/R-induced
liver and lung injuries causing histopathological damages was evaluated as prescribed. The lung injury score was evaluated as grades ranging from 0 (minimum) to 4 (maximum), of each of the following 3 terms: accumulation of the inflammatory cells, alveolar wall thickness, and alveolar hemorrhage [37]. The liver histological score was evaluated as grades ranging from 0 (minimum) to 4 (maximum), of each of the following 6 terms: condensation of the nucleus, fragmentation of the nucleus, nuclear fading, cytoplasmic color fading, vacuolization, and erythrocyte stasis [38].

### 2.5. Biochemical Analysis

The levels of tissue ALT, AST, and MPO were measured according to the manufacturer’s instructions (Nanjing, Jiangsu, China). The protein samples of randomly 3 individual rat lung and liver tissues were loaded and resolved using 8, 10, or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then, the proteins were transferred to nitrocellulose membranes for 1 h, and at the solution of 5% skimmed milk, the membranes were blocked for an additional 1 h at 37°C. After that, they were incubated with the primary antibody overnight at 4°C: Cox-2, IL-1β, IL-6, TNFR-2, caspase-3, caspase-9, Bcl-2, Bax, p53, NF-κB p65, NF-κB p50, IK-α, and IKKβ (Proteintech, Wuhan, Hubei, China). Then, the membranes were washed in TBS and stained with DAPI (1 μg/mL) for an additional 1 h at 37°C. After that, the slides were washed with PBS. And slides were stained using a TUNEL staining kit (One-Step TUNEL kit®, Nanjing, Jiangsu, China) according to the manufacturer’s instruction. The slides were visualized using a fluorescence microscope (BX63, IX81, Olympus, Japan).

### 2.7. Immunostaining of TUNEL

Rat lung and liver tissue slides were dewaxed with gradients of alcohol, then permeabilized with 0.1% TritonX-100 solution for 10 min; after that, the slides were washed with PBS. And slides were stained using a TUNEL staining kit (One-Step TUNEL kit®, Nanjing, Jiangsu, China) according to the manufacturer’s instruction. The slides were visualized using a fluorescence microscope (BX63, IX81, Olympus, Japan).

### 2.8. Western Blotting

The protein samples of randomly 3 individual rat lung and liver tissues were loaded and resolved using 8, 10, or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then, the proteins were transferred to nitrocellulose membranes for 1 h, and at the solution of 5% skimmed milk, the membranes were blocked for an additional 1 h at 37°C. After that, they were incubated with the primary antibody overnight at 4°C: Cox-2, IL-1β, IL-6, TNFR-2, caspase-3, caspase-9, Bcl-2, Bax, p53, NF-κB p65, NF-κB p50, IK-α, and IKKβ (Proteintech, Wuhan, Hubei, China). Then, the membranes were washed in TBS and stained with DAPI (1 μg/mL) for an additional 1 h at 37°C. After that, the slides were washed with PBS. And slides were stained using a TUNEL staining kit (One-Step TUNEL kit®, Nanjing, Jiangsu, China) according to the manufacturer’s instruction. The slides were visualized using a fluorescence microscope (BX63, IX81, Olympus, Japan).
Figure 3: Cinnamaldehyde ameliorated against mesenteric I/R-mediated lung and liver inflammation. (a) Tissue levels of pulmonary and hepatic MPO. (b) The levels of IL-6, IL-1β, Cox-2, and TNFR-2 protein expression in lung tissues. (c) Inflammatory protein expression quantifications in lung tissues. (d) The levels of IL-6, IL-1β, Cox-2, and TNFR-2 protein expression in liver tissues. (e) Inflammatory protein expression quantifications in liver tissues. All results are analyzed as the mean ± SD (n = 3). ***P < 0.001, **P < 0.01, and *P < 0.05 vs. sham group; ###P < 0.001, ##P < 0.01, and #P < 0.05 vs. I/R group.
37°C in secondary antibody (Proteintech, Wuhan, Hubei, China) for 1 h. Enhanced chemiluminescent (ECL) solution (Proteintech, Wuhan, Hubei, China) was used to visualize the membranes. And proteins were quantified by using Image Lab software (Bio-Rad, CA, USA) for 3 independent experiments. β-Actin was the corresponding expression of normalization [39, 40].

2.9. Statistical Analysis. One-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test was used to the normal distribution data. All values are presented as the mean ± standard deviation (SD), of at least 3 independent experiments. Prism 5.0 (GraphPad, La Jolla, CA) software was used for data analysis. P values of less than 0.05 indicated the statistical significance of the differences.

3. Results

3.1. Protective Effects of CA against Mesenteric I/R-Induced Lung and Liver Morphological Damages. The following are the pulmonary and hepatic morphological damages induced by mesenteric I/R injury in I/R rats compared with sham rats using H&E staining: characterized with significant inflammatory cell infiltration, perivascular and interstitial edema, deposition in the alveolar spaces, and hemorrhage in lung tissues (Figure 2(a)) and nuclear condensation, cell shrinkage, and margination and apoptotic debris in liver tissues (Figure 2(b)). And these morphological alterations were significantly ameliorated by CA pretreatment at concentrations 10 and 40 mg/kg (Figures 2(a) and 2(b)). The pulmonary and hepatic histopathological scores were significantly increased in injured rats by mesenteric I/R compared with the...
Figure 5: Continued.
Lung and Liver Apoptosis.

3.3. Protective Effects of CA against Mesenteric I/R-Induced Lung and Liver Apoptosis. Our results indicated that TUNEL-positive apoptotic cells were significantly more observed in both injured pulmonary and hepatic tissues of the mesenteric I/R group compared with the sham groups. And pretreatment with CA significantly decreased TUNEL-positive cells in lung and liver tissues compared with I/R-injured rats (Figures 4(a) and 4(b)), suggesting that pretreatment with CA significantly alleviated mesenteric I/R-induced lung and liver apoptosis. CA did not significantly show TUNEL-positive cells in lung and liver tissues in sham rats.

3.4. The Role of CA-Induced Protection against p53 and NF-κB

3.4.1. Cinnamaldehyde Inhibits p53. The transcriptional factor p53 is a proapoptotic factor which exerts a crucial role in mediating apoptosis in lung and liver injuries [41, 42]. The results of western blot showed that the expression of apoptosis proteins, such as caspase-3, caspase-9, Bax, and p53, was significantly increased and the antiapoptotic protein expression of Bcl-2 was significantly decreased in both lung and liver tissues of mesenteric I/R rats compared with the sham rats. The pretreatment with CA restored the increased expression of caspase-3, caspase-9, Bax, and p53 and also restored the reduced Bcl-2 significantly in both pulmonary and hepatic tissues of mesenteric I/R-injured rats, suggesting that CA ameliorated mesenteric I/R-induced lung and liver apoptosis (Figures 5(a)–5(d)). CA did not significantly show any changes in the apoptotic protein expressions in sham rats.

3.4.2. Cinnamaldehyde Inhibits NF-κB. The transcriptional factor NF-κB is related to inflammation, immune responses, oxidative stress, and cell death in injured tissues [43–45]. The main NF-κB complex family member is NF-κB p65 [46]. Western blot results showed that NF-κB p65, NF-κB p50, IK-α, and IKKβ protein expressions of NF-κB-related proteins were significantly changed in both injured pulmonary and hepatic tissues of the mesenteric I/R group compared with the sham groups. And pretreatment with CA significantly decreased TNFα, IL-1β, and IL-6 protein expressions (Figures 3(b)–3(e)), suggesting that pretreatment with CA significantly alleviated mesenteric I/R-induced injuries through attenuating the inflammation. CA did not significantly change any of the above protein expressions in sham rats.
signaling pathway were upregulated in both injured pulmonary and hepatic tissues of mesenteric I/R rats significantly in comparison with the normal tissue controls, and CA significantly downregulated these protein expressions in both injured pulmonary and hepatic tissues of mesenteric I/R rats (Figures 6(a)–6(d)), indicating that CA pretreatment protects...
against mesenteric I/R-induced lung and liver inflammation and apoptosis. CA did not show any significant protein expression alterations in sham groups.

3.4.3. Cinnamaldehyde Protects against Mesenteric I/R-Triggered p53 and NF-κB p65 Nuclear Translocation in Lung and Liver Tissues. The transcriptional factors NF-κB p65 and p53 are both activated under stress conditions, inducing NF-κB p65 and p53 subunit import into the nucleus and triggering the inflammatory mediators and proapoptotic targets [47, 48]. The immunofluorescence assay showed that the p53 nuclear import was more increased in both injured lung and liver tissues of I/R rats compared with the sham animals. And pretreatment with CA inhibited the nuclear translocation of p53 significantly in both injured lung and liver tissues of I/R rats (Figures 7(a) and 7(b)), suggesting the role of p53 in mediating CA-induced protection against mesenteric I/R-induced pulmonary and hepatic injuries.

The immunofluorescence results also showed that NF-κB p65 nuclear import was also significantly increased in both injured lung and liver tissues in mesenteric I/R rats in comparison with the control groups. And pretreatment with CA inhibited the nuclear translocation of NF-κB p65 significantly in both lung and liver of I/R-injured rats (Figures 8(a) and 8(b)), suggesting the way of NF-κB p65 in mediating CA-induced protection against mesenteric I/R-induced pulmonary and hepatic injuries.

4. Discussion

Mesenteric I/R induces either local [49–51] or remote organ injuries, including heart [52], lung [53], liver [54], kidney [55], and brain [56] injuries. Remote organ injuries are a
serious consequence of mesenteric I/R injuries due to the damage of intestinal mucosa and translocation of bacteria and endotoxins into the distant body organs [57, 58].

Our previous study showed that mesenteric I/R induced excessive intestinal (local) morphological changes, inflammation, oxidative stress, apoptosis, and upregulated p53 and NF-κB pathway-related proteins in mesenteric I/R-injured rats and hypoxia/reoxygenation- (H/R-) injured intestinal epithelial cells-6 (IEC-6), and CA pretreatment significantly restored all the above-mentioned changes in mesenteric I/R-treated rats and H/R-treated IEC-6 cells [18].

In this study, our results supported our proposal. CA pretreatment alleviated morphological damages in both injured lung and liver tissues of mesenteric I/R rats and significantly restored the injury-related enzymatic alterations of ALT and AST in liver tissues. CA pretreatment significantly attenuated inflammation in mesenteric I/R-induced lung and liver injuries via downregulating the expression of inflammation-related proteins, including IL-6, IL-1β, Cox-2, and TNFR-2, and by reversing MPO activity in both injured lung and liver tissues of mesenteric I/R rats.

The transcriptional factors p53 and NF-κB were involved in various tissue injuries [59–62]. NF-κB p65 is the key subunit of the NF-κB pathway (NF-κB p65, NF-κB p50, IK-α, and IKKβ) which creates a crucial role in inducing apoptosis, immune response, and inflammation [63, 64]. And p53 is also the key subunit of the p53 signaling pathway [65]. Our results showed that NF-κB-related proteins, including NF-κB p65, were significantly downregulated in both lung and liver tissues of CA-pretreated rats compared to I/R-treated rats.
kB p65, NF-κB p50, IK-α, and IKKβ, were significantly upregulated, and p53-related proteins, including caspase-3, caspase-9, Bax, and p53, were also significantly upregulated, and Bcl-2 was significantly downregulated, in both lung and liver tissues of mesenteric I/R-injured rats. Pretreatment with CA restored these aberrant parameters significantly in both lung and liver tissues of mesenteric I/R-injured rats. Pretreatment with CA showed a significant reduction of TUNEL-apoptotic cells in both injured lung and liver tissues of mesenteric I/R rats, suggesting CA-mediated attenuation of inflammation and apoptosis against mesenteric I/R-induced lung and liver injuries. The immunofluorescence assay showed that CA mediated a significant inhibition of both NF-κB p65 and p53 nuclear translocation in both injured lung and liver tissues of mesenteric I/R rats, indicating that attenuation of inflammation and apoptosis and inhibition of nuclear translocation were related to CA-mediated amelioration against mesenteric I/R-induced lung and liver injuries.

Infection and inflammation are among the major clinical challenges in the treatment of mesenteric I/R injury and are involved in the recommendations for the management and treatment of mesenteric ischemia (acute or chronic) in the recent clinical guidelines [66–70], indicating that both anti-infection and anti-inflammatory interventions are required for treatment of mesenteric I/R-induced local and/or remote organ injuries [71–73]. Although the antibacterial properties of antibiotics are necessary for the treatment of mesenteric I/R, they often cause renal and hepatic injuries [74–76]. Current evidence indicates that CA not only possess ameliorative effects and anti-inflammatory effects for ameliorating tissue injuries but also possess antibacterial activities [25, 77, 78], suggesting that CA could be a potential therapeutic intervention for treating mesenteric I/R-induced local and/or remote organ injuries.

In conclusion, in this study, our results revealed that pretreatment with CA significantly ameliorated and protected against mesenteric I/R-induced lung and liver injuries via reducing aberrant inflammation and apoptosis. CA did not show any significant changes on the corresponding controls. And CA-mediated protection and amelioration against mesenteric I/R-induced remote organ injuries via suppression of p53 and NF-κB exert an important role in the protection. Based on the ameliorative effects together and its bacterial inhibitory effects, this study reveals that CA can be considered a potential choice for alleviating mesenteric I/R-induced remote organ injuries, and the actual relationship of p53 and NF-κB under I/R and remote organ injuries may require future study.

**Abbreviations**

| Acronym | Description |
|---------|-------------|
| I/R:    | Ischemia-reperfusion |
| H/R:    | Hypoxia/reoxygenation |
| CA:     | Cinnamaldehyde |
| SMA:    | Superior mesenteric artery |
| AMI:    | Acute mesenteric artery |
| ig:     | Intragastric gavage |
| ip:     | Intraperitoneally |

| Acronym | Description |
|---------|-------------|
| AST:    | Aspartate transaminase |
| ALT:    | Alanine transaminase |
| H&E:    | Hematoxylin and eosin |
| MPO:    | Myeloperoxidase |
| TNFR-2: | Tumor necrosis factor receptor type-2 |
| Cox-2:  | Cyclooxygenase-2 |
| IL-6:   | Interleukin-6 |
| IL-1β:  | Interleukin-1β |
| NF-κB:  | Nuclear factor kappa B |
| IK-α:   | I kappa B alpha |
| IKKβ:   | Inhibitor of nuclear factor kappa-B kinase subunit beta |
| p53:    | Tumor protein p53 |
| Bax:    | Bcl-2-associated X protein |
| Bcl-2:  | B-cell lymphoma 2 |
| PBS:    | Phosphate-buffered saline |
| SPD:    | Sprague-Dawley |
| IEC-6:  | Intestinal epithelial cells-6 |
| SDS-PAGE: | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| T-TBS:  | Tween-20 and Tris-buffered saline |
| ECL:    | Enhanced chemiluminescent |
| ANOVA:  | Analysis of variance |
| SD:     | Standard deviation |
| DAPI:   | 4',6-Diamidino-2-phenylindole |
| BCA:    | Bicinchoninic acid |
| TUNEL:  | Terminal deoxynucleotidyl transferase dUTP nick end labeling |

**Data Availability**

The data used to support the findings of this study are available from Marwan Almoiliqy, Pengyuan Sun, and Yuan Lin upon request.

**Additional Points**

**Highlights.** (1) Intestinal ischemia/reperfusion-induced lung injury. (2) Intestinal ischemia/reperfusion-induced liver injury. (3) Intestinal ischemia/reperfusion-induced lung and liver inflammation. (4) Intestinal ischemia/reperfusion-induced lung and liver apoptosis. (5) Cinnamaldehyde protects against intestinal ischemia/reperfusion-induced lung and liver inflammation and apoptosis. (6) Cinnamaldehyde protects against intestinal ischemia/reperfusion-induced lung and liver injuries via inhibition of NF-κB and p53 signaling pathways.

**Conflicts of Interest**

The authors declare no conflicts of interest.

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