Apigenin attenuates acute myocardial infarction of rats via the inhibitions of matrix metalloprotease-9 and inflammatory reactions

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Abstract: Acute myocardial infarction (AMI) is the myocardial necrosis caused by coronary artery acute and persistent ischemia and hypoxia. Matrix metalloprotease-9 (MMP-9) plays an important role in a series of process of occurrence and development of AMI. Inflammatory reaction plays the key role in all kinds of damage factors in AMI. Apigenin (API) has effectively restrained the activity of MMP-9, anti-inflammatory and hepatic fat oxidizing properties. API significantly improved AMI of rats through inhibiting MMP-9 and inflammatory reactions in a few recent studies. Our investigation detected the infarct size of AMI rats, casein kinase (CK), the MB isoenzyme of creatine kinase (CK-MB) and lactate dehydrogenase (LDH) and cardiac troponin T (cTnT) activities in AMI rats were also analyzed with commercial kits. Additionally, Nuclear factor kappa B (NF-κB), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) levels of whole bloods of AMI rats were also detected using commercial kits. Next, MMP-9 protein of cardiac in AMI rats was measured with gelatin zymography assays. Finally, caspase-3 and caspase-9 activities in AMI rats were analyzed with commercial kits. In the present study, our work indicated API might significantly reduce the infarction size of AMI rat. It was shown that the treatment of API could decrease the expression of MMP-9 level and reduce the activities of NF-κB, TNF-α, IL-1β and IL-6 in AMI rats. Next, API treatment could reduce caspase-3 and caspase-9 activities and decrease cellular apoptosis of AMI rats. Our findings concluded that API ameliorates acute myocardial infarction of rats via inhibiting MMP-9 and inflammatory reactions.

Keywords: Apigenin, acute myocardial infarction, MMP-9, inflammatory, apoptosis

Introduction

It was well established that acute myocardial infarction (AMI) is caused by persistent ischemia and hypoxia, finally leading to many clinical cases of severe and persistent pain behind the sternum [1]. It is a very common disorder in Western countries [2]. In recent years, there is a rising trend in China. It was estimated that the new morbidity quantity in each year was increased to at least 500,000 [3].

It is well known that matrix Metalloproteases (MMPs) contain at least 26 members and can adjust and control the synthesis and degradation of extracellular matrix. Prior work illustrated that it was involved in the reconstruction of ventricular [4]. MMP-9 is the important members of matrix metalloproteinases, which is secreted by vascular smooth muscle cells and the expression of them is significantly increased in atherosclerotic plaque, contributing to the pathogenesis of AMI [5].

Inflammatory reaction is closely involved in the generation of AMI. Under normal conditions, inflammatory cells and vascular endothelial cells do not attach or hardly attach. But after stimulation by inflammatory factor, adhesion molecule can be quickly activated and help the inflammatory cell to be attached and accumulated in the endothelial cells, subsequently releasing the inflammatory mediators to damage the endothelial cells [6]. Three kinds of selectins of adhesion molecules were found to be related to the adhesion and rolling of inflammatory cells in vascular endothelial and the accumulation of damaged region [7].

It was previously reported that API possessed diverse pharmacological actions including anti-tumor, anti-oxidative stress, anti-DNA damage
Apigenin; Acute myocardial infarction

and so on [8]. Furthermore, API was found to remarkably suppress metastasis via MMP-9 pathway in ovarian tumor model [9]. However, up to date, there is no relevant publication on the effect of API against AMI. Our present work aimed to evaluate the protective potential of API against AMI and further figure out its potential molecular mechanism.

Materials and methods

Experimental animals

Sixty male Wistar rats, weighing 220-250 g, were supplied from the Experimental Animal Center of Second Hospital of Hebei Medical University. All surgical procedures have been approved by Second Hospital of Hebei Medical University and performed in strict accordance with the related ethical standards.

Drug administration

API (Sigma, with a purity of 95%) was dissolved in physiological saline. Casein kinase (CK), the MB isoenzyme of creatine kinase (CK-MB), lactate dehydrogenase (LDH) and cardiac troponin T (cTnT) commercial kits were from Sangong Biotech (Shanghai, China). Nuclear factor kappa B (NF-κB), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) commercial kits were purchased from JianCheng Bioengineering (Nanjing, China). BCA protein assay kit was supplied from Tiangen (Beijing, China). Caspase-3 and Caspase-9 Activities Assay Kit were bought from Sangong Biotech (Shanghai, China).

Preparation of acute myocardial infarction and group design

AMI model was prepared as previously described [10]. Successful establishment of all rats AMI models was verified by regional cyanosis of myocardial surface and represented by ST-segment elevation. All experimental rats were randomly divided into sham group (n = 10), Vehicle group (n = 10) and API treatment group (n = 10) (various doses of API: 10 mg/kg, 20 mg/kg and 40 mg/kg, respectively). Rats of sham group were treated identically except for no ligation. Rats of vehicle group were administered with the equal volume of physiological saline. Physiological saline and API were injected once a day. Rats were operated on by occlusion of coronary artery after the last administration at 30 minutes.

Infarct size measurement

After ligation for 6 h, the hearts from different groups were carefully dissected and 2 mm thick sections was sliced for infarct size measurement. Infarct size of rats was determined with 1% 2, 3, 5-triphenyltetrazolium chloride (1.5%; Sigma-Aldrich Co.) at 37°C for 30 min in the dark [11, 12]. The area of heart without color was deemed as the ischemic heart muscles, but the area with brick red was considered as normal myocardium. The infarct size area was calculated by the volume and weight as a percentage of the left ventricle.

Determinations of CK, CK-MB, LDH and cTnT

Serum samples of rats were obtained from vena cava after ligation for 6 h. The CK, CK-MB, LDH and cTnT activities in rats were detected by respective commercial kits (Sangong Biotech, Shanghai, China).

Measurement of NF-κB, TNF-α, IL-1β and IL-6 levels

After 6 h ligation, whole bloods of rats from different groups were collected and a commercial ELISA kit was employed to measure the serum levels of NF-κB, TNF-α, IL-1β and IL-6 in rats following the manufacturer’s instruction.

Measurement of MMP-9

After API treatment, MMP-9 activities were detected by gelatin zymography assays. For short, 50 µg samples were absorbed into the new centrifuge tube and then an equal volume of sodium dodecyl sulfate (SDS) sample buffer were added into the centrifuge tube. The miscible liquids were subjected to 10% SDS-PAGE.
Apigenin; Acute myocardial infarction

**Table 1.** The activities of CK, CK-MB, and LDH, the level of cTnT in a rat model of AMI (mean ± S.D., n = 10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK (U/mL)</th>
<th>CK-MB (IU/L)</th>
<th>LDH (U/L)</th>
<th>cTnT (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.21 ± 0.02</td>
<td>83.11 ± 7.11</td>
<td>1713.11 ± 311.27</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.78 ± 0.05**</td>
<td>212.35 ± 7.19**</td>
<td>5747.39 ± 437.62**</td>
<td>0.67 ± 0.04**</td>
</tr>
<tr>
<td>API (10 mg/kg)</td>
<td>0.38 ± 0.02**</td>
<td>113.15 ± 7.48**</td>
<td>3135.26 ± 335.62*</td>
<td>0.23 ± 0.05**</td>
</tr>
<tr>
<td>API (20 mg/kg)</td>
<td>0.31 ± 0.04**</td>
<td>95.03 ± 7.29**</td>
<td>2678.84 ± 316.26**</td>
<td>0.19 ± 0.04**</td>
</tr>
<tr>
<td>API (40 mg/kg)</td>
<td>0.29 ± 0.03**</td>
<td>85.66 ± 8.23**</td>
<td>2367.89 ± 324.21**</td>
<td>0.16 ± 0.02**</td>
</tr>
</tbody>
</table>

**P<0.01 vs. sham group, #P<0.05, ##P<0.01 vs. vehicle group. Sham, sham-operated; Vehicle, vehicle-treated; API (10 mg/kg)-treated; API (20 mg/kg)-treated; API (40 mg/kg)-treated groups.

**Figure 2.** API decreases infarct size in a rat model of AMI (n = 10, mean ± S.D.). **##P<0.01 versus vehicle group.

**Figure 3.** API inhibits MMP-9 in a rat model of AMI (n = 10, mean ± S.D.). MMP-9 activity was dose-dependently reduced after exposure to API for 24 h by Gelatin zymography assays. **P<0.01 versus sham group; #P<0.01 versus vehicle group.

Caspase-3 and caspase-9 activities assay

In order to detect the apoptotic state during AMI, caspase-3 and caspase-9 activities were measured by corresponding Assay Kits (Sangong Biotech, Shanghai, China). The data was expressed as the percentage of the control group.

Statistics

Data were expressed as means ± S.D. and analyzed by SPSS 17.0 software. Differences between experimental groups were analyzed using ANOVA analysis. A p value of less than 0.05 were deemed statistically significant.

Results

**API suppresses the activities of CK, CK-MB, and LDH, the level of cTnT in a rat model of AMI**

The chemical structure of API was displayed in Figure 1. It was obviously found that the activities of CK, CK-MB, LDH and cTnT were significant increased in the serum, compared with the sham controls (Table 1). When treatment with API (10, 20 and 40 mg/kg) for 24 h, the remarkably reduced levels of CK, CK-MB, LDH and cTnT were observed in serum of AMI rat model, compared to that in the vehicle group (Table 1).

**API decreases the Infarct size in AMI rat model**

We then investigated whether the effect of API on the infarction size of AMI rat. After treatment with API (10, 20 and 40 mg/kg) for 24 h, the
infarction size were significantly reduced, compared to the vehicle group (Figure 2).

**API inhibits MMP-9 in a rat model of AMI**

Next we aimed to analyze whether API might affect MMP-2 production in AMI rat model. We found that the activity of MMP-9 in vehicle group was markedly increased, compared to the sham group (Figure 3). However, API treatment dose-dependently reversed this phenomenon in rats with AMI, compared to the vehicle group.

**API suppresses the activities of TNF-α, IL-1β, IL-6 and NF-κB in AMI rat model**

It was remarkably found that inflammatory cytokines including NF-κB, TNF-α, IL-1β and IL-6 were evidently activated during AMI, as illustrated in Figure 4A-D. Treatment with API (10, 20 and 40 mg/kg) for 24 h could obviously reduce the inflammatory mediators (NF-κB, TNF-α, IL-1β and IL-6), compared to the vehicle group.

**API inhibits the activities of caspase-3 and caspase-9 in a rat model of AMI**

To confirm the protection of API against AMI, caspase-3 and caspase-9 activities were analyzed in our present study. We found that caspase-3 and caspase-9 activities in vehicle group were evidently increased, compared to the sham group (Figure 5A, 5B). It was noted that caspase-3 and caspase-9 activities were remarkably suppressed in API-treated AMI group.

**Discussion**

The major findings of our present work depicted that API, the well-known medicinal herb, has effective role against AMI in rats and further...
Apigenin; Acute myocardial infarction

illustrated its protection might be associated with inhibiting MMP-9 activity and inflammatory reactions.

MMPs is normally secreted by myocardial cells, vascular endothelial cells, smooth muscle cells, foam cells and other cells, including the protease family that depends on Ca²⁺ and Zn²⁺. Current work shows that MMPs is the protease to adjust and control extracellular matrix, which can promote the degradation of ECM, accelerate the progression of atheromatous plaque and lead to the formation of unstable plaque [13, 14]. MMP-9 can reflect the status of atherosclerotic plaque and plays an important role in a series of process of generation of AMI [15]. Our study disclosed that API treatment significantly suppressed MMP-9 activity in rats subjected to AMI. In fact, prior work illustrated that API could inhibit metastasis of orthotopic ovarian tumor and down-regulate MMP-9, which was in agreement with our findings [9].

After AMI, the inflammatory factors such as TNF-α, IL-1β and IL-6 were reported to be elevated and their rising degree was closely related to the disease severity and prognosis of the patients [16]. Meanwhile, in atherosclerosis, NF-κB could activate TNF, IL-1, IL-8 and other inflammatory factors [17]. Therefore, activation of NF-κB is also likely to be participated in the pathogenesis of AMI. Our study revealed that API could obviously reduce the activities of NF-κB, TNF-α, IL-1β and IL-6 in AMI rats. It was previously found that API evidently alleviated cutaneous inflammation in murine models of acute dermatitis [18]. Besides, API was also found to weaken inflammatory reaction following UVB-induced skin inflammation [19]. In the meantime, our present work also showed that API treatment could reduce caspase-3 and caspase-9 activities in AMI rat models. Consistently, API was also shown to have protection against liver ischemia-reperfusion injury in rats [20].

In conclusion, it was for the first time to delineate that API could alleviate AMI injury and decrease the infarction size in AMI rats. Meanwhile, further investigation disclosed that the protection of API against AMI rat models was linked with inhibiting MMP-9 activity and inflammatory reactions.

Disclosure of conflict of interest

None.

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