Thrombosis Research 131 (2013) e270–e273

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Effects of quercetin on LPS-induced disseminated intravascular coagulation (DIC) in rabbits

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ARTICLE INFO

Article history:
Received 4 October 2012
Received in revised form 16 February 2013
Accepted 1 March 2013
Available online 6 April 2013

Keywords:
Quercetin
DIC
LPS
anticoagulation
anti-inflammation

ABSTRACT

Introduction: Quercetin is widely distributed in plants and has been reported to have effects of anti-inflammation and anti-thrombosis. In this study, we evaluated the protective effect of quercetin on LPS-induced experimental DIC in rabbits, and tried to clarify its mechanism against DIC.
Materials and Methods: LPS-induced DIC model in rabbits was established through continuous infusion of 100 ug · kg^{-1} · h^{-1} LPS for a period of 6 h. Six groups were divided: quercetin-treated groups (0.5, 1.0, and 2.0 mg · kg^{-1} · h^{-1}, respectively), LPS-control group, heparin-control group (100 IU · kg^{-1} · h^{-1}), and saline-control group. APTT, PT, and plasma FIB level were measured, the plasma levels of ALT, BUN, and TNF-α were detected, and the activity of Protein C and ATIII was recorded.
Results: A continuous injection of LPS induced a gradual impairment of hemostatic parameters, a rise in plasma level of TNF-α, and damage in renal and hepatic function. The intravenous administration of quercetin significantly attenuated the increase of APTT, PT, ALT, BUN, and TNF-α, and the decrease of plasma FIB level and activity of Protein C and ATIII.
Conclusion: Quercetin may have a protective effect against LPS-induced DIC in rabbits through anti-inflammation and anticoagulation.

Introduction

Disseminated intravascular coagulation (DIC) is an acquired syndrome secondary to a variety of underlying disorders, characterized by the elevated levels of inflammatory factors and the systemic overactivation of coagulation with subsequent intravascular fibrin deposition in the microvasculature and impaired hemostatic parameters [1]. Disturbance of the microcirculation and organ failure caused by numerous microthrombi is a serious complication and a cause for the poor prognosis of DIC [2,3]. The basic pathological mechanism of DIC includes the excessive release of inflammatory factors such as TNF-α and the spread of microvascular thrombosis [1,4]. According to the guideline of the International Society for Thrombosis and Haemostasis (ISTH) [5], in addition to treatment of underlying disease, there are four approaches of DIC management, including blood component therapy, antifibrinolytic agents, and anticoagulants, including heparin, antithrombin and recombinant human activated protein C (rhAPC). Among the three phases of DIC, the first phase where microthrombi form has the highest mortality rate, only if we inhibit formation of microthrombi, could we relatively decrease multi-organ failure and mortality rate. Thus, anticoagulants are relatively more accepted and clinically used in DIC. According to ISTH guidelines, heparin and rhAPC are most commonly used. Their effects in DIC may be due to a combination of anticoagulation and anti-inflammation. However, heparin does not obviously decrease mortality rate and leads to bleeding tendency. RhAPC cannot be used in DIC patients presenting with bleeding or at high risk of bleeding, and it appears to be only effective in higher disease severity cases. Thus, further investigations for new anticoagulants with both anti-inflammatory and anticoagulant effects to act against DIC are needed.

There is a long history in Traditional Chinese Medicine of the use of many plants or their extracts to treat diseases. Flavonoids are widely distributed in plants and are reported to have anti-inflammatory [6,7] and anti-thrombotic [7] effects. Quercetin is a flavonoid abundantly existing in Camellia sinensis, capers and lovage. Previous studies have revealed that quercetin could suppress release of TNF-α [8], and inhibit

Abbreviations: LPS, lipopolysaccharide; DIC, disseminated intravascular coagulation; APTT, activated partial thromboplastin time; PT, prothrombin time; FIB, fibrinogen; ALT, alanine aminotransferase; BUN, blood urea nitrogen; TNF-α, tumor necrotic factor-α; ATIII, antithrombinIII.

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http://dx.doi.org/10.1016/j.thromres.2013.03.002

platelets aggregation in vitro [9] and in vivo [10]. These characteristics raise the possibility of its potential use in DIC.

Although many of the properties of quercetin are known, there are few studies investigating its effect on DIC. In this study, we determined the effect of quercetin on a LPS-induced DIC model in rabbits, and investigated the mechanism of its action in an attempt to gain a better understanding of its clinical potential.

Materials and Methods

Reagents

Quercetin (3,3′,4′,5,6-Pentahydroxy flavone), LPS (from E. coli), and heparin were purchased from Sigma (St Louis, USA). The solution of quercetin and LPS was newly prepared before use. The reagent packs for the activity of ATIII and protein C were obtained from Sun Biotechnology Company (Shanghai, China). TNF-α ELISA kit was purchased from Rapid Bio Lab (Calabass, USA). The other reagents were of analytical grade from commercial sources.

Animals

Adult male New Zealand white rabbits (weight 2–2.5 kg, Grade II) were supplied by the Medical Experimental Animal Center of Guangzhou Province, China.

Experimental models and drug treatments

All procedures were conducted according to the ethical guidelines of National Guide for the Care and Use of Laboratory Animals and were approved by Ji-nan University Animal Care and Use Committee (Guangzhou, China).

Animals were anesthetized by an intravascular injection of 30 mg · kg^{-1} sodium pentobarbitol through the marginal ear vein of rabbits, followed by intraperitoneal supplements of 30 mg sodium pentobarbitol given 2 h, 4 h, and 6 h individually after the initiation of infusion. DIC experimental models were established according to the method performed by Hermida J [11], by infusing LPS in 60 ml of saline solution at a rate of 100 ug · kg^{-1} · h^{-1} (10 ml · h^{-1}) through the ipsilateral marginal ear vein over a period of 6 h.

Treatments started simultaneously with LPS infusion through the contralateral marginal ear vein. Six different groups were established, each contained 10 animals. Over a period of 6 h, quercetin treatment groups (low, medium, and high doses of quercetin) were given 0.5 mg · kg^{-1} · h^{-1} (low-dose group), 1.0 mg · kg^{-1} · h^{-1} (medium-dose group), and 2.0 mg · kg^{-1} · h^{-1} (high-dose group) quercetin respectively in 60 ml saline solution at a rate of 10 ml · h^{-1}; LPS-control group was infused with saline solution at a rate of 10 ml · h^{-1}; heparin-control group was infused with heparin at a rate of 100 IU · kg^{-1} · h^{-1} (10 ml · h^{-1}); the additional rabbits in saline-control group, which were given neither LPS nor quercetin, were infused with 60 ml saline solution (10 ml · h^{-1}) through both marginal ear veins.

Sample collection and handling

For each rabbit, 1 ml of blood sample was attained through a catheter inserted into a carotid artery before infusion, 2 h after infusion, and 6 h after infusion, respectively. The blood samples were collected immediately in 3.8% sodium citrate (1:10 vol/vol citrate/blood), centrifuged at the rate of 3000 r/min for 10 min, and stored at −80 °C until assayed.

Laboratory methods

The activity of Protein C and ATIII was measured according to the reagent pack instruction based on chromogenic substrates. Automatic analyzers (Sysmex SF-8000, Japan) were used to determine APTT, PT, and FIB. Another automatic analyzer (HITACHI 7600, Japan) was used to detect plasma levels of ALT and BUN. The concentration of TNF-α was determined using ELISA kit.

Data analysis

Differences between data of groups were evaluated for significance using student t test. Repeated measures analysis of variance was used for multivariate analysis. Data at 2 h and 6 h were converted to percentages, with a value of 100% assumed for basal data and were expressed as mean ± SD. Differences with *P* values less than 0.05 were considered to be statistically significant.

Results

LPS-induced DIC

Both 2 h and 6 h after LPS initiation (100 ug · kg^{-1} · h^{-1}), there were significant increases in APTT, PT, and plasma levels of TNF-α, BUN and ALT, accompanied with obvious decreases in FIB level and the activity of Protein C and ATIII ($P < 0.05$, when compared with the saline-control group, seen in Figs. 1–6 and Table 1).

Effects of heparin on LPS-induced DIC

2 h post-heparin infusion, there were significant attenuation of APTT, PT and plasma levels of TNF-α, ALT and BUN, no obvious improvement of FIB level and activity of Protein C and ATIII ($P < 0.05$, when compared with the LPS-control group, seen in Figs. 1–6 and Table 1). While 6 h post-infusion, except FIB level, all the other parameters were improved ($P < 0.05$, when compared with the LPS-control group, seen in Figs. 1–6 and Table 1).

Effects of quercetin on LPS-induced DIC

2 h after drug treatment, the aberrance of APTT, TNF-α and FIB levels were significantly attenuated by all the three doses of quercetin, PT was improved by the low and medium doses of quercetin, BUN and ALT were improved by the medium and high doses of quercetin, and activity of Protein C was improved by the high dose of quercetin ($P < 0.05$, when compared with LPS-control group, seen in Figs. 1–6 and Table 1). Whereas 6 h after drug treatment, the abnormal prolongation of APTT, the plasma levels of FIB, TNF-α, BUN and ALT, and the activity of Protein C and ATIII were improved by all doses of quercetin, while the pathological prolongation of PT was only ameliorated by the

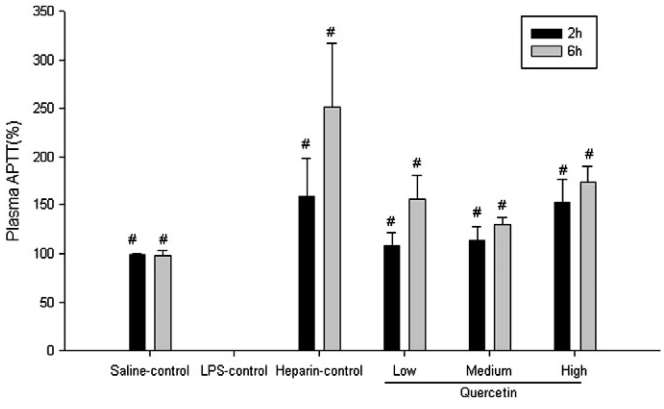

Fig. 1. Plasma APTT 2 h and 6 h after LPS administration into rabbits in different groups. Note: The APTT in LPS-control group was so high (the data was more than 1000) that it exceeds the capability of the automatic analyzers (Sysmex SF-8000, Japan) used in this study. #P < 0.01, compared with the LPS-control group.