Electrocardiographic and Biochemical Evidence for the Cardioprotective Effect of Antioxidants in Acute Doxorubicin-Induced Cardiotoxicity in the Beagle Dogs

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Doxorubicin (DOX) is a potent antitumor agent, but the cardiotoxicity mediated by the formation of reactive oxygen species limit its clinical use. The present study aims to explore electrocardiographic and biochemical evidence for the cardioprotective effect of two antioxidants, Lycium barbarum polysaccharides (LBP, the main antioxidant in Lycium barbarum) and edaravone (a potent free radical scavenger, EDA) against DOX-induced acute cardiotoxicity in beagle dogs. In this study, male beagle dogs received daily treatment of either LBP (20 mg/kg, per os (p.o.)) or EDA (2 mg/kg, intravenously (i.v.)) for 7 d and then followed by an intravenous injection of DOX (1.5 mg/kg). DOX (15 mg/kg) significantly induced acute cardiotoxicity in dogs characterized by conduction abnormalities (including decreased heart rate, ST segment elevation, QT intervals prolongation, inverted T wave, arrhythmia, and myocardial ischemia) and increased serum creatine kinase (CK) and aspartate aminotransferase (AST). Pretreatment with LBP or EDA effectively alleviated both DOX-associated conduction abnormalities and increased serum CK and AST. Moreover, physiological and serum biochemical evidences demonstrated that EDA is more effective than LBP in alleviating these abnormalities produced by DOX in heart. All these results confirm and extend previous observations in rats concerning the effectiveness of LBP or EDA against DOX-induced cardiomyopathy.

Key words doxorubicin; Lycium barbarum polysaccharide; edaravone; cardiotoxicity; antioxidant

Doxorubicin (DOX), a quinone-containing anthracycline antineoplastic, is used for the treatment of solid and hematopoietic tumors. However, its dose-dependent cardiotoxicity hampered its clinical application.1) Recent studies have suggested that DOX-induced cardiotoxicity involves the formation of reactive oxygen species (ROS) and amplification of mitochondrial dysfunction.1,2) Moreover, the anti-cancer effects of DOX do not follow the identical mechanisms of ROS. The majority of strategies to protect cardiomyocytes against DOX-induced oxidative injury in heart therefore focused on administering antioxidants in the past.1) A number of antioxidants, such as lycopene, N-acetylcysteine and vitamin E were proved to ameliorate the DOX-induced cardiac cell damage without compromising its anti-tumor efficacy in the rats or mice model.3–5) However, most of them were tried with limited success in preventing DOX-associated cardiotoxicity in large-sized animals such as dogs or pigs.6–9)

Lycium barbarum, a famous Chinese medicinal herb, has a long history of use as an antioxidant and to promote sexual fertility. Lycium barbarum polysaccharides (LBP), consisting of various botanic polysaccharide including arabinose, rhamnose, xylose, mannose, galactose and glucose, are the most important functional constituents in red-colored fruits Lycium barbarum. LBP and edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, a potent free radical scavenger, EDA) were found to elicit a typical cardioprotective effect against DOX-related oxidative stress in rats.7,8) However, no similar cardioprotective effect of either LBP or EDA was reported in beagle dogs. Electrocardiography (ECG) is one of the standard methods used to assess cardiac function and is often performed to evaluate whether cardioprotective agents would improve DOX-induced conduction abnormalities.9,10) Moreover, the usefulness of biochemical indicators such as serum creatine kinase (CK) and aspartate aminotransferase (AST) in assessment of DOX-associated cardiotoxicity in experimental animals has also been indicated by various studies.11) The present study aims to explore electrocardiographic and biochemical evidence for the cardioprotective effect of those two antioxidants in DOX-induced acute cardiotoxicity in beagle dogs.

MATERIALS AND METHODS

Chemicals DOX was obtained from Pfizer Italia S.r.l. (Nerviano, Italy) as a 10 mg/bottle lyophilized powder. It was dissolved in 20 ml of 0.9% saline for injection. Lycium Chinese mill Polysaccharide was purchased from Zhejiang

Fig. 1. Experimental Protocol

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Fangge Pharmaceutical Industry Co., Ltd. (Qingyuan, China). The polysaccharide content was >60.0%, as determined by sulfuric acid-anthrone method. The edaravone injection was purchased from Nanjing Simcere Dongyuan Pharmaceutical Co., Ltd. (Nanjing, China).

**Animals Protocol** Male beagle dogs weighing between 10—12 kg, were obtained from Zhejiang Jiaxing Institute of Experimental Animal. Dogs were housed with temperature range of 16—26 °C and humidity range of 40—70%, and fed with commercial diets and tap water ad libitum throughout the experimental period. All animal experiments were carried out in accordance with Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council and promulgated by the State Science and Technology Commission of China and the guideline for animal experiments of Zhejiang Academy of Medical Sciences. The animals were randomly divided into three groups (n=4 per group) to receive different treatment procedures. As shown in Fig. 1, LBP group received LBP plus DOX and EDA group received EDA plus DOX, whereas the DOX group was given DOX only. LBP (20 mg/kg) was orally administered and EDA (2 mg/kg) was intravenously (i.v.) injected daily for 21 continuous days. DOX (1.5 mg/kg) was i.v. injected at day 7. General appearance and mortality were observed and recorded daily. Blood samples (3 ml) were taken to analyze the biochemical markers and the ECG was tracked to investigate the change in cardiovascular function at 0, 1, 2, 3, 4, 5, 6, 7, and 14 d after the DOX injection.

**Electrocardiogram Recording** ECGs were recorded with subcutaneous electrodes in fully awake dogs and amplified by Softron ECG Processor SP2000 (Softron Co., Ltd., Tokyo, Japan).

**Determination of Biochemical Indicators in Serum** Enzyme activities of alanine transaminase (ALT), AST and CK in serum were measured using an autoanalyzer (Model 7020, Hitachi Medico, Japan).

**Statistical Analysis** Chi-square tests were performed to analyze the categorical data. All the other data were expressed as means±S.E.M. and analyzed by one-way analysis of variance (ANOVA) and Tukey’s HSD test. A probability of error (p<0.05) was selected as the criterion of statistical significance.

**RESULTS**

**General Toxicity** At the end of the treatment period, all dogs remained alive, but they showed scruffy fur, weak and weight-lost. The mean weight of the dogs in DOX, LBP+DOX, and EDA+DOX groups were similar, being 9.1±0.4, 8.5±0.5, and 9.3±0.6 kg, respectively.

**Electrocardiogram** ECG is one of the standard technologies used to monitor and assess cardiac function, so the ECG tracings were recorded for evaluating heart rate and rhythm disorders. As shown in Fig. 2, dosing LBP or EDA daily has no significant effect on the durations of the electrocardiographic parameters. The heart rate was similar in DOX (138.8±8.1 bpm), LBP+DOX (146.3±9.8 bpm), and EDA+DOX groups (145.8±6.1 bpm) before DOX induction. DOX injection led to significant decrease in the mean heart rate. However, pretreatment with EDA or LBP partly improved DOX-induced bradycardia. The statistical
results indicated that pretreatment with EDA effectively increased the heart rate especially from 5 d posterior to DOX injection. Pretreatment with LBP had similar effect, though the difference of heart rate between DOX and LBP/DOX group didn’t reach the statistical significance. As shown in Fig. 3 and Table 1, dogs treated with DOX showed several ECG abnormalities including ST segment elevation, QT intervals prolongation, inverted T wave, arrhythmia and myocardial ischemia. Chi-square analysis suggested that pretreatment with EDA or LBP significantly compromised DOX-induced ST segment elevation (p<0.05) but had little effect on QT prolongation. EDA treatment is also effective to ameliorate DOX-induced arrhythmia and myocardial ischemia (p<0.05); however, no statistically significant difference was found in rhythm disorders or ischemia between the LBP/DOX group and the DOX group.

**Serum Biochemistry** Because DOX triggers the disruption of cardiac myocytes and the release of intracellular CK and AST into serum, serum CK and AST rather than ALT are cardiac-specific markers.11) AST and ALT are important markers of liver injury, so the ratio of AST to ALT is always used to distinguish that AST is resourced from hepatic or cardiac injury. Those three indicators were examined in the present study. As shown in Fig. 4, pretreatment with LBP and EDA for several days did not significantly affect serum ALT, AST and CK and the levels of those three indicators in three groups were comparable before DOX injection. AST, ALT and CK levels were increased by DOX administration which peaked in 24—48 h and dropped to baseline in nearly 3 d after DOX injection. The ratio of AST to ALT was 3.1±1.7 at day 1 compared to 0.7±0.2 at day 0 in DOX group, which, together with the increased serum AST and CK, suggested that treatment with DOX induced obvious cardiac injury.

It is EDA but not LBP that was effective in decreasing DOX-induced ALT increase within 4 d after DOX injection. Compared with AST in DOX group, pretreatment with EDA or LBP significantly dropped DOX-induced AST level (p<0.05). Similar to the effect on AST, the decrease in AST/ALT ratio appeared at day 1 in DOX-induced dogs pretreated with antioxidant, though the difference of AST/ALT ratio between DOX group and LBP or EDA groups didn’t reach the statistical significance. DOX treatment alone induced nearly 5 times of increases in CK level at 24 h after injection compared with that at day 0. Pretreatment with LBP

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**Table 1. Effects of Treatment with LBP or EDA on Electrocardiography Parameters in DOX-Treated Dogs**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DOX</th>
<th>LBP + DOX</th>
<th>EDA + DOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST segment elevation</td>
<td>Normal</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>Changed</td>
<td>27</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Changed/total</td>
<td>0.84</td>
<td>0.28*</td>
<td>0.56*</td>
</tr>
<tr>
<td>QT prolongation</td>
<td>Normal</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>Changed</td>
<td>11</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Changed/total</td>
<td>0.34</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Myocardial ischemia</td>
<td>Normal</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>Changed</td>
<td>15</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Changed/total</td>
<td>0.47</td>
<td>0.28</td>
<td>0.09*</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>Normal</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Changed</td>
<td>10</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Changed/total</td>
<td>0.31</td>
<td>0.31</td>
<td>0.06*</td>
</tr>
</tbody>
</table>

* Significantly different (p<0.05) from respective values in the DOX group.

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**Fig. 4. Effect of Pretreatment with Lycium barbarum Polysaccharides (LBP) or Edaravone (EDA) on Serum Levels of Alanine Aminotransferase (ALT), Creatine Kinase (CK) and Aspartate Aminotransferase (AST) in DOX-Injected Beagle Dogs (n=4 in Each Group)**

All readings are means±S.E.M. * Significantly different (p<0.05) from respective values in DOX group. ♦, DOX group; ■, LBP + DOX group; ●, EDA + DOX group.
(20 mg/kg) significantly prevented the increase of CK activity by DOX exposure in beagle dogs at day 1 \((p=0.036)\), where 2 mg/kg of EDA were comparatively more effective in the prevention \((p=0.011)\).

**DISCUSSION**

The clinical utility of DOX is marred by an increased risk of myocardial injury and congestive heart failure, which is mainly caused by free radicals from DOX disposition.\(^1,2\) The quinone metabolism may lead to the generation of \(O_2^-\) and highly reactive metabolites, such as \(\cdot\)OH and \(H_2O_2\), which result in cell damage \(via\) lipid peroxidation, protein cross-linking, and DNA fragmentation.\(^12,13\) As cardiomyocytes have relatively lower level of antioxidant enzymes \((e.g.,\ SOD\ and\ glutathione-peroxidase)\), cardiac tissue is more susceptible to oxidative damage.\(^6\) Fortunately, the antioxidant effects of DOX do not follow the identical mechanisms of oxidative injury.\(^3\) By understanding these facts, two antioxidants, LBP and EDA, had been proved to alleviate DOX-induced acute cardiotoxicity in the rats.\(^7,8\) The present study investigated the cardioprotective effect of LBP and EDA in DOX-induced acute cardiotoxicity in the beagle dogs.

As traditional Chinese medicine, *Lycium barbarum* has been shown to have antioxidant activity both \(in\ vitro\) and \(in\ vivo.\)^14 Our previous study also proved that pretreatment with LBP (200 mg/kg), the main antioxidant \(in\ Lycium barbarum\), significantly attenuated DOX-induced cardiac myofibrillar disarrangement in rats. LBP was also effective in decreasing the levels of serum CK to improve conduction abnormalities significantly attenuated DOX-induced cardiac myofibrillar disarrangement in rats. LBP was also effective in decreasing the levels of serum CK to improve conduction abnormalities caused by DOX exposure. Moreover, the protective effect of LBP against DOX-induced acute cardiotoxicity was parallels with its activity in reducing oxidative stress, whereas LBP does not attenuate the anti-tumor activity of DOX.\(^7\) EDA, a free radical scavenger, is used to rescue acute cerebral infarction by scavenging the free radicals.\(^15\) EDA can scavenge \(\cdot\)OH and other free radicals, and further inhibits \(OH\)-dependent lipid peroxidation.\(^15\) Other’s study showed that EDA \((3\ mg/kg)\) effectively prevented DOX-induced chronic cardiac deterioration.\(^9\)

In agreement with other findings,\(^9,10\) the acute cardiotoxicity of DOX \((1.5\ mg/kg)\) in dogs was characterized by conduction abnormalities \((including\ decreased\ heart\ rate,\ ST\ segment\ elevation,\ QT\ intervals\ prolongation,\ inverted\ T\ wave,\ arrhythmia,\ and\ myocardial\ ischemia)\ and increased serum CK and AST. The serum CK and AST reached apogee at 24 h and dropped to baseline in 48—72 h after myocardial damage. Pretreatment with EDA was more effective than LBP in dropping DOX-induced AST and CK level. However, ECG tracings showed that the conduction abnormalities and arrhythmia were chronic reaction followed by sluggish recovery in DOX-treated dog, which was different from that in rats wherein the conduction abnormalities have an acute drop and reach a nadir at 24 h in rats after DOX treatment.\(^9\) LBP or EDA pretreatment effectively alleviated DOX-associated arrhythmia and ST elevation. Moreover, the physiological and serum biochemical evidences proved that EDA is more effective than LBP in alleviating these cardiac abnormalities produced by DOX exposure in the heart.

There are two possible pathways to explain the protective effects of LBP against DOX-induced cardiac injury. One way is that LBP directly removed reactive oxygen species and suppressed lipid peroxidation of cardiac cells. The other way is that LBP indirectly scavenged the free radicals by activating antioxidant enzyme systems in the heart tissues.\(^16\) The pathway to protect against DOX-induced cardiotoxicity by EDA is similar to that of LBP, including quenching of active oxygen and inhibition of hydroxyl radicals-induced lipid peroxidation. In addition, EDA can also scavenge other free radicals such as the peroxynitrite radical.\(^15\) Together with evidence of LBP or EDA’s protective effect on DOX-relative cardiotoxicity in rats, these studies suggest that the antioxidant property of LBP or EDA may account for its protective effects in DOX-induced cardiotoxicity in dogs.

In summary, electrocardiographic and biochemical evidences indicate that pretreatment with LBP or EDA effectively alleviated DOX associated conduction abnormalities and increased serum CK and AST. These results confirm and extend previous observations in rats concerning the effectiveness of LBP or EDA against DOX-induced cardiomyopathy. Due to the fact that the protective effect against DOX-induced cardiotoxicity of many antioxidants in rodents were limited in non-rodents, our results would be of great importance for LBP or EDA as clinical agent in antitumor chemotherapy.

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**REFERENCES**


