Can the Antioxidant, Ginkgo Biloba Extract and the Protease Inhibitor, Aprotinin, Protect Rats Against Leiurus Quinquestriatus Scorpion Venom-Induced Cardiovascular Effects?

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INTRODUCTION

Leiurus Quinquestriatus, known as the yellow scorpion, Toxicon 1995; 33: 825-858. indicates that the severity of scorpion envenoming, attributed mainly to cardiovascular and respiratory manifestations (2,3), have necessitated the necessity of fully understanding the pathological mechanisms involved. Although much is known about scorpion venom-elicited cardiovascular changes, cellular mechanisms including the role of free radicals and oxidative stress need further investigation. Mekij et al. (4) and Fatani et al. (5) demonstrated an increase in markers indicative of free radical generation and increased lipid peroxides following scorpion envenomation, indicating the possible involvement of oxidative stress and peroxidation of lipids in the venom-induced damages. Oxidative stress has been implicated in several diseases such as hyperlipidemia, cardiac dysrythmias, and myocardial damage (6), all of which are observed following scorpion envenomation (7,8). Extensive evidence indicates that antioxidants decrease the risk of cardiovascular diseases, stabilize membranes and prevent their functional damage (7,8). On the other hand, Fatani et al. (9) suggested the possible involvement of proteins in venom-elicited cardiovascular manifestations. Thus, it would be interesting to discover if an antioxidant or a protease inhibitor would be useful in ameliorating LQV-elicited cardiovascular manifestations.

AIM OF THE WORK

The aim of this study was to test the effectiveness of the antioxidant, ginkgo biloba extract (G), and the protease inhibitor, aprotinin (Ap), in protecting anesthetized rats from venom-induced cardiovascular and respiratory changes. This may help determine whether oxidative stress or proteases are involved in the cardiovascular damage evoked by the venom, and if antioxidants and protease inhibitors may have a future role in the treatment of the scorpion envenoming syndrome.

MATERIALS & METHODS

Anesthetized (urethane, 1.75 g kg-1, i.v.) and heparinized (1000 UI kg-1, i.v.) male Winter rats (180-200 g) were prepared for blood pressure and electrocardiographic recordings (10).

1. Mean arterial blood pressure (MABP) was measured via a polyethylene cannula (G2) inserted into the carotid artery and connected to a pressure transducer & physiograph (Harvard, UK).
2. Heart rate (HR) was measured from electrocardiographic tracings obtained via s.c. needle electrodes attached to an electrocardiograph (Cardiomax, Japan). Heart rate (HR) and MABP were monitored in all animals (5/group) until death or up to 5 hr after venom injection in surviving rats, which were then killed by diethyl ether overdose.
3. After a 30 min stabilization period following surgical procedures, animals were injected with LQV venom alone (0.3 mg kg-1, i.v.) or 30 min after aprotinin (4000 K.I.U. kg-1, i.p.) and/or on the last day of a two-week daily treatment with standardized ginkgo biloba leaf extract (150 mg kg-1, p.o.).
4. Lung edema index was calculated. (Lung weight body weight x 100)

Table 1: Lung body weight index in anesthetized rats injected with Leiurus quinquestriatus (LQV) venom alone or after pretreatment with ginkgo biloba extract and/or aprotinin

<table>
<thead>
<tr>
<th>Group/Dose(s) injected</th>
<th>Lung/body weight index</th>
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<tbody>
<tr>
<td>Group 1 (Normal saline, Control)</td>
<td>0.78±0.011</td>
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<tr>
<td>Group 2 (LQV Venom)</td>
<td>0.86±0.027</td>
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<tr>
<td>Group 3 (Ginkgo)</td>
<td>0.73±0.025</td>
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<tr>
<td>Group 4 (LQV Venom+Aprotinin)</td>
<td>0.71±0.024</td>
</tr>
<tr>
<td>Group 5 (LQV Venom+Ginkgo+Aprotinin)</td>
<td>0.69±0.036</td>
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Animals were injected with LQV venom alone (0.3 mg kg-1, i.v.), or after pretreatment with ginkgo biloba extract (150 mg kg-1, p.o., daily for two weeks) or/and aprotinin (4000 K.I.U. kg-1, i.p.). Control groups were injected with 0.9% NaCl. Five rats were used in each group. See methods for further details. Lung body weight index = lung weight/total body weight X 100. Values are significantly different from control group at *P<0.05, or from venom-treated group at +P<0.01, ++P<0.001.

RESULTS

Figure 1: Effects of Leiurus quinquestriatus (LQV) venom alone or after pretreatment with ginkgo biloba extract and/or aprotinin on (A) heart rate (HR) and (B) mean arterial blood pressure (MABP) of anesthetized rats (n=5) & vertical lines ± SEM. Refer to METHODS for further details. Note the greater effectiveness of aprotinin in combating venom-induced changes.

Figure 2: Effects of pretreatment with ginkgo biloba extract (G) and/or aprotinin (Ap) on LQV-venom-evoked initial bradycardia and maximum heart rate reached, plus (B) the concomitant initial maximum fall and subsequant rise in MABP of anesthetized rats (n=5) & vertical lines ± SEM. Refer to METHODS for further details. Values are significantly different from LQV-treated group at *P<0.05, ++P<0.01 and LQV at *P<0.05, ++P<0.01.

Figure (3): Survival distribution function curves of rats injected with LQV venom alone or after pretreatment with ginkgo biloba extract (G) and/or aprotinin (Ap). Y axis: survival distribution, (O) death & (I) survival, X-axis: survival time (min). All groups, n=5. Refer to methods for details. Animals treated with saline or treatments alone survived. Note the greater efficacy of pretreatment with Ap in prolonging survival (P<0.01 vs venom alone, Covariance survival statistics).

CONCLUSION

In general, the protease inhibitor, aprotinin, and to a lesser extent the antioxidant, standardized ginkgo biloba leaf extract appeared effective in delaying venom-induced terminal hypertension plus bradycardia and prolonging survival. They were each alone and in a greater degree than the combination of decreasing the enhanced lung/body weight index. This would indicate the involvement of oxidative stress and proteases in venom-elicited changes in the cardiovascular and respiratory systems.

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REFERENCES