Ameliorating the anticancer drug "Adriamycin" acute Cardiotoxicity by Rosuvastatin and Telmisartan in rats

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Abstract:
Adriamycin, an anthracycline antibiotic is a powerful antineoplastic drug, but its therapeutic usefulness is limited by its cardiotoxicity. The present study investigated the effects of pretreatment with rosuvastatin and telmisartan alone or in combination against adriamycin induced cardiotoxicity in rats using biochemical approaches. The animals were divided into eight groups of 5 animals each. The first group received no drug(s) po but a single dose of distilled water (7.5 ml/kg, ip) at day eight, which serves as the control group. The second group received no drug(s) po but a single dose of adriamycin (15 mg/kg, ip) at day eight, and serves as adriamycin only received group. The third and sixth group received rosuvastatin (2 , 10) mg/kg/day respectively for nine days, and on day eight, one hour after drug administration, a single dose of adriamycin (15 mg/kg, ip) was given. The fourth and seventh group received telmisartan (2 , 4) mg/kg/day respectively for nine days, and at day eight, one hour after drug administration, a single dose of adriamycin (15 mg/kg, ip) was given.

The fifth and eighth group received both drugs, where the fifth group Received both of rosuvastatin (2 mg/kg, po) and telmisartan (2 mg/kg, po), 1 hour apart, daily for nine days, and on day eight, one hour after drug administration, a single dose of adriamycin (15 mg/kg, ip) was given. While the eighth group received both of rosuvastatin (10 mg/kg, po) and telmisartan (4 mg/kg, po), 1 hour apart, daily for nine days, and on day eight, one hour after drug administration, a single dose of adriamycin (15 mg/kg, ip) was given.

At day ten of the study, blood samples were taken for biochemical analysis. Rats treated with adriamycin showed cardiotoxicity as evidenced by elevation of serum lactate dehydrogenase (LDH) activity, serum malondialdehyde (MDA) level, cardiac troponin (CTn-I) level and interleukine 17(IL-17) level. Pretreatment with rosuvastatin and telmisartan alone or in combination elicited a significant decrease in the activities of all markers measured in comparison with adriamycin treated group.

These results suggest that both rosuvastatin and telmisartan treatment provides a significant protective effect against acute-adriamycin induced cardiotoxicity in rats.

Keyword: Adriamycin, Cardiotoxicity, Rosuvastatin, Telmisartan

Introduction:
Adriamycin is considered as one of the most powerful and effective chemotherapeutic approved drugs of the Food and Drug Administration and has been found to have broad anticancer action (Carvalho et al., 2009). Adriamycin exerted its activity mainly by intercalation with DNA and by this means it inducing damage to the DNA and inhibiting the synthesis of macromolecules that are essential to maintain cell life (Gharanei et al., 2013). The successful use of adriamycin has been hindered by its most important and common “cardiotoxic” adverse effect which remains the major limitation of its use with strong impaction on life quality and survival. Anthracyclines induce cardiotoxicity by a cumulative and dose dependent manner leading to myocardial damage that ranging from mild disturbance of cardiac biomarkers to irreversible cardiomyopathy (Zhang et al., 2009). Since adriamycin is necessary in cancer treatment, protection against its cardiotoxicity represents an important challenge to prevent detrimental effects on the heart functions while maintaining the same anticancer efficacy, from this point of view many trials have taken place in an attempt to reduce the adriamycin induced cardiotoxicity. (Chatterjee et al., 2010).

Rosuvastatin considered as one of the best lipid lowering drugs. Previous studies demonstrated the cardioprotective effects of rosuvastatin against many type of cardiac injury...
by mechanisms other than its effects on lipid (Taylor et al., 2013).

Telmisartan is an angiotensin receptor blocker. Recent studies showed that beside its excellent role in controlling blood pressure, it has variant beneficial touch on the myocardium that also related to large extent to its ability to counteract harmful intracellular signaling induced by angiotensin-AT1 receptor interaction (Kruzliak et al., 2013).

In the present study an attempt was made to assess the influence of pretreatment with rosuvastatin and telmisartan alone or in combination in different doses on adriamycin induced acute cardiotoxicity in rats.

Materials and Methods:

This study is conducted in The Iraqi Center for Cancer and Medical Genetic in cooperation with the Department of Pharmacology, College of Medicine at Al-Mustansiriya University in Baghdad, Iraq during 2014. Forty Sprague Dawley male rats where enrolled in this study. Their ages ranged from 4-7 weeks with body weights ranged from 100 to 200 gram. The rats were housed in cages and kept at 25 °C with artificial 12 hours light-dark cycle. The rats allowed to had laboratory chow pellet and to drink tap water ad libitum. They were left for two weeks without interference for acclimatization. They had no manifestation of any illness upon examination.

After two weeks of acclimatization period, the animals were randomly divided into 8 groups, 5 rats in each group and assigned as I, II, III, IV, V, VI, VII and VIII where all groups are sacrificed at day ten.

Group I: Received no drug(s) po but a single dose of distilled water (7.5 ml/kg, ip) at day eight, which serves as the control group.

Group II: Received no drug(s) po but a single dose of adriamycin (15 mg/kg, ip) at day eight, which serves as adriamycin only received group.

Group III: Received rosuvastatin (2 mg/kg, po), daily for nine days, and at day eight a single dose of adriamycin (15 mg/kg, ip) was given.

Group IV: Received telmisartan (2 mg/kg, po), daily for nine days, and at day eight a single dose of adriamycin (15 mg/kg, ip) was given.

Group V: Received both of rosuvastatin (2 mg/kg, po) and telmisartan (2 mg/kg, po) 1 hour apart, daily for nine days, and at day eight a single dose of adriamycin (15 mg/kg, ip) was given.

Group VI: Received rosuvastatin (10 mg/kg, po), daily for nine days, and on day eight a single dose of adriamycin (15 ml/kg, ip) was given.

Group VII: Received telmisartan (4 mg/kg, po), daily for nine days, and at day eight a single dose of adriamycin (15 mg/kg, ip) was given.

Group VIII: Received both of rosuvastatin (10 mg/kg, po) and telmisartan (4 mg/kg, po), 1 hour apart, daily for nine days, and at day eight a single dose of adriamycin (15 mg/kg, ip) was given.

The rats were anesthetized using chloroform, blood samples were collected by intracardiac puncture in sterile labeled tubes, then centrifuged and stored in the freezer (-20 °C) to be assessed later. Serum cardiac troponin-I (CTn-I), malondialdehyde (MDA) and interleukin-17 (IL-17) were determined using specific ELISA kit for each. Moreover Serum lactate dehydrogenase (LDH) was determined using ready-made kit.

Data analysis was done by using IBM SPSS (statistical package for social sciences) version 20. The data was expressed as mean ± standard deviation (SD). The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means or ANOVA test for difference among more than two independent means.

Results:

At the end of the study, serum CTn-I level was significantly increased (P < 0.05) in the adriamycin only received group (204.12 ± 15.08) as compared with the control group (28.27 ± 15.20) while rosuvastatin alleviates this destructive effect through lowering CTn-I levels significantly (p < 0.05) for both 2 mg/kg dose and 10 mg/kg dose.

The significant increase of the serum CTn-I level in adriamycin only received group is also modified by using telmisartan which decreased the levels of CTn-I significantly (p < 0.05) at both 2 mg/kg and 4 mg/kg dose.

Regarding treatment with a combination of the two drugs, the results demonstrate a significant reduction in the CTn-I levels (p < 0.05) at both low and high doses when compared with adriamycin only received group.

The changes in CTn-I levels are summarized in figure 1 and table 1.

Figure 1: Comparison among experimental groups means of serum CTn-I level where the one star refers significant difference regarding group 1 (control) and the two stars refer significant difference regarding group 2 (adriamycin only received group).
Serum lactate dehydrogenase level was elevated significantly (p < 0.05) in adriamycin only received group (271.1 ± 77.94) as compared with the control group (41.9 ± 11.82) but the treatment with rosuvastatin improves this damaging effect and this is reflected by lowering LDH levels significantly (p < 0.05) for both 2 mg/kg and 10 mg/kg dose. Administration of telmisartan also decreases the levels of LDH significantly (p < 0.05) in both 2 mg/kg dose and 4 mg/kg dose. Combination therapy with both rosuvastatin and telmisartan results in lowering LDH level significantly (p < 0.05) at both low and high doses when compared with adriamycin only received group.

The changes in LDH levels are summarized in figure 2 and table 1.

This study revealed that there was a significant increase in serum MDA level (P < 0.05) in the adriamycin only received group (177.99 ± 135.2) in comparison with the control group (33.16 ± 11.17) but the pretreatment with rosuvastatin ameliorates this harmful effect noticed by lowering MDA levels significantly (p < 0.05) for both 2 mg/kg dose and 10 mg/kg dose. Telmisartan has also an advantageous effect appeared by decreasing the levels of MDA significantly (p < 0.05) in both 2 mg/kg dose and 4 mg/kg dose. Administration of both rosuvastatin and telmisartan in combination significantly reduce MDA levels significantly (p < 0.05) at both low and high doses in comparison with adriamycin only received group.

The changes in MDA levels are summarized in figure 3 and table 1.

Level of serum IL-17 was significantly increased (P < 0.05) in the adriamycin only received group (248.69 ± 67.945) as compared with the control group (50.27 ± 16.94) while pretreatment with rosuvastatin improves this damaging effect evidenced by lowering IL-17 levels significantly (p < 0.05) for both 2 mg/kg dose and 10 mg/kg dose. Administration of both rosuvastatin and telmisartan in combination significantly (p < 0.05) in both 2 mg/kg dose and 4 mg/kg dose.

Concerning treatment with a combination of rosuvastatin and
telmisartan, it also results in reducing levels of IL-17 significantly (p < 0.05) at both low and high doses when compared with adriamycin only received group. The changes in IL-17 levels are summarized in figure 4 and table 1.

Cardiac injury is the main limiting factor for the use of adriamycin as an anticancer agent, the cardiotoxicity of adriamycin is attributed to complex mechanisms that include oxidative stress, intracellular calcium dysregulation, mitochondrial damage, and apoptosis/necrosis. This study investigates the effects of pretreatment with two famous cardioprotector agents namely rosuvastatin and telmisartan, which were used in different doses alone or in combination, on acute cardiotoxicity induced by adriamycin.

This study revealed clearly the adriamycin induced myocardial injury represented by a significant increase in plasma level of CTn-I (p < 0.05) in adriamycin treated rats as compared with control group that shown in figure (1), the results of the present study are in agreement with several published experimental studies that have characterized the response of CTn-I to chemotherapy induced acute myocardial injury (Herman et al., 1998; Bertinchant et al., 2003). It’s well-known that cardiac troponin is the best and gold standard biomarker for myocardial injury and cardiotoxicity (O’Brien, 2008). It is only released into the plasma when cardiac myocytes injured, so a rise in troponin during treatment with adriamycin reflects its acute cardiotoxicity (Cardinal et al., 2002).

The present study also shown that adriamycin induce myocardial injury represented by a significant increase in serum LDH (p < 0.05) in adriamycin treated rats as compared with control group that demonstrated in figure (2), the results of

Table 1: Biomarkers means in all experimental groups in the study where the one star refers significant difference regarding group 1 (control) and the two stars refers significant difference regarding group 2 (adriamycin only received group).

<table>
<thead>
<tr>
<th>Groups</th>
<th>CTn-I</th>
<th>LDH</th>
<th>MDA</th>
<th>IL-17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>28.27 ± 15.20**</td>
<td>41.9 ± 11.82**</td>
<td>33.16 ± 11.17**</td>
<td>50.27 ± 16.94**</td>
</tr>
<tr>
<td>Group 2</td>
<td>204.12 ± 15.08*</td>
<td>271.1 ± 77.94*</td>
<td>177.99 ± 135.2*</td>
<td>248.69 ± 67.945*</td>
</tr>
<tr>
<td>Group 3</td>
<td>114.56 ± 54.65* &amp;***</td>
<td>176.75 ± 23.73* &amp;***</td>
<td>47.01 ± 42.86* &amp;***</td>
<td>104.58 ± 26.35* &amp;***</td>
</tr>
<tr>
<td>Group 4</td>
<td>80.37 ± 20.05* &amp;***</td>
<td>145.95 ± 38.24* &amp;***</td>
<td>60.01 ± 36.65* &amp;***</td>
<td>127.89 ± 35.57* &amp;***</td>
</tr>
<tr>
<td>Group 5</td>
<td>68.57 ± 23.37*</td>
<td>142.56 ± 35.65* &amp;***</td>
<td>61.27 ± 9.87* &amp;***</td>
<td>125.37 ± 11.25* &amp;***</td>
</tr>
<tr>
<td>Group 6</td>
<td>89.14 ± 37.76* &amp;***</td>
<td>83.07 ± 23.32*</td>
<td>47.38 ± 18.97*</td>
<td>109.07 ± 32.23* &amp;***</td>
</tr>
<tr>
<td>Group 7</td>
<td>76.65 ± 21.55* &amp;***</td>
<td>72.68 ± 17.85*</td>
<td>45.82 ± 22.11*</td>
<td>109.93 ± 25.75* &amp;***</td>
</tr>
<tr>
<td>Group 8</td>
<td>76.48 ± 15.52* &amp;***</td>
<td>71 ± 12.43*</td>
<td>49.4 ± 17.37*</td>
<td>116.65 ± 10.79* &amp;***</td>
</tr>
</tbody>
</table>

**P value**

| P value | P<0.05 | P<0.05 | P<0.05 | P<0.05 |

Discussion:

Cardiac injury is the main limiting factor for the use of adriamycin as anticancer agent, the cardiotoxicity of adriamycin is attributed to complex mechanisms that include oxidative stress, intracellular calcium dysregulation, mitochondrial damage, and apoptosis/necrosis. This study investigates the effects of pretreatment with two famous cardioprotector agents namely rosuvastatin and telmisartan, which were used in different doses alone or in combination, on acute cardiotoxicity induced by adriamycin.

This study revealed clearly the adriamycin induced myocardial injury represented by a significant increase in plasma level of CTn-I (p < 0.05) in adriamycin treated rats as compared with control group that shown in figure (1), the results of the present study are in agreement with several published experimental studies that have characterized the response of CTn-I to chemotherapy induced acute myocardial injury (Herman et al., 1998; Bertinchant et al., 2003). Its well-known that cardiac troponin is the best and gold standard biomarker for myocardial injury and cardiotoxicity (O’Brien, 2008). It is only released into the plasma when cardiac myocytes injured, so a rise in troponin during treatment with adriamycin reflects its acute cardiotoxicity (Cardinal et al., 2002).

The present study also shown that adriamycin induce myocardial injury represented by a significant increase in serum LDH (p < 0.05) in adriamycin treated rats as compared with control group that demonstrated in figure (2), the results of
The results of this study pointed out that rats treated with rosuvastatin against environmental factors that interfere with oxidative stress and the cardiac inflammatory response, including the release of pro-inflammatory cytokine following adriamycin treatment. One of these cytokines involved is TNF-α, which mediate myocardial damage (Bien et al., 2007). IL-17 as mentioned earlier has been found to up-regulate TNF-α, IL-1β and, IL-6 (Dragan et al., 1998) and up regulation NF-kB through TRAF6 which acts as a signal transducer in the NF-kB pathway through activation of (IKK) (Hirahara et al., 2001). The present study may explain the ability of statins to attenuate other cytokines like (TNF- α), (IL-6) and (NF-kB) reported by the previous studies, at least partially through attenuation of IL-17 level. Some experimental studies suggested that anti-inflammatory effect of statins in myocardium can be related to the stimulation of PPARγ and inhibition of NF-kB expression in myocardial tissue (Shen et al., 2010) or inhibition of NF-kB activation by (TNF- α) by preventing signaling in the RhoA pathway (Xu et al., 2006). Sheng et al, 2005 reported that treatment with statin up-regulate the expression of PPARα and PPARγ and lowered the mRNA expression of MMP9, MMP2 and IL-1β. Telmisartan showed significant reduction of Ctn-1 in treated groups (in both doses 2mg/kg and 4mg/kg) after injection of a single dose of adriamycin(p < 0.05) as compared with adriamycin only treated group which demonstrated in figures (1) and (2). This reflects that telmisartan ameliorates the cardiac injury in the treated groups.

Our results support the earlier researches (Toko et al., 2002; Muzzaffar et al., 2008 ) that confirmed the important role of AT1- mediated pathway in adriamycin induced cardiotoxicity.

MDA level was significantly decreased after telmisartan pre-treatment at both (low and high doses) were the (p < 0.05) as compared with adriamycin only treated group which illustrated in figure (3), this effect of telmisartan can be explained by both antioxidant and anti-inflammatory properties of telmisartan which are related in part to its inhibition of assembly of NADPH oxidase onto cell membranes, a process that is initiated by Ang II binding to AT1-R leading to activation of Rac-1 pathway (Welch, 2008).
Telmisartan pre-treatment at both (2 mg/kg and 4 mg/kg doses) decreased the IL-17 levels significantly as shown in figure (4) and the (p < 0.05), the anti-inflammatory properties of telmisartan are again related in part to its inhibition of assembly of NADPH oxidase onto cell membrane as mentioned, moreover the activation of PPAR-γ by telmisartan promotes catalase gene expression while inhibits NF-κB, thus fighting the oxidative stress and down regulating many of pro-inflammatory responses (Blessing et al., 2008). Furthermore PPAR-γ activation down regulates the transcription of genes of inflammatory cytokines like TNF-α, growth factors, adhesion molecules, iNOS genes and chemotactic factors (Qing-ping et al., 2009).

In the present study it has been found that pre-treatment with both of rosuvastatin and telmisartan in combination result in significant decrease in CTn-I, LDH, MDA and IL-17 (p < 0.05) as compared with adriamycin alone treated group which shown in figures (1), (2), (3) and (4). Moreover pre-treatment with small dose combination of both drugs were as cardio protector as large dose combination. Finding of this study in this regard can be explained by the sharing in means through which each of rosvastatin and telmisartan exert their influence against adverse effects of adriamycin in rat cardiac tissue where many previous studies declared that both of rosvastatin and telmisartan have antioxidant and anti-inflammatory properties that are, to some extent, inter-related between them at the molecular levels.

In the present study use of both drugs in combination resulted in non-significant lowering of all studied biomarkers in comparison to use of each drug alone and this can be attributed either to the high similarity and limited capacity of molecular mechanisms through which both drugs counteract the oxidative stress and inflammatory process, or due to small sample size of each studied group. The later explanation to non-significant cardioprotective effects of pre-treatment of both drugs when used alone versus their use in combination may be of greater concern especially if the finding of recent study, which state that there is a synergistic reducing effect of IL-17 after using a combination of telmisartan-rosuvastatin in hypertensive patients with carotid atherosclerosis, is taken in consideration (Liu et al., 2014).

**Conclusion:**

We can conclude that adriamycin in a dose of 15 mg/kg induces acute cardiotoxicity manifested by significant increase in the biomarkers related to cardiac damage (CTn-I and LDH), oxidative stress (MDA) and inflammation (IL-17), furthermore this study is supporting the role of pro inflammatory cytokine IL-17 in pathology of adriamycin induced cardiotoxicity.

Pretreatment with rosvastatin and telmisartan alone or in combination can ameliorate the cardiotoxic effects of adriamycin evidenced by a significant reduction in the measured biomarkers therefore they have therapeutic value in acute adriamycin induced cardiotoxicity.

**References:**

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تقليل سمية القلب الناتجة من استخدام مضاد السرطان الأدرياميسين بواسطة الروزوفاستاتين والتلمسارين

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الخلاصة

أجريت الدراسة الحالية لتقييم التأثير العلاجي لاستخدام الروزوفاستاتين والتلمسارين في التقليل من سمية القلب الناتجة عن الأدرياميسين في الجرذان المختبرية باستخدام الطرق الكيميائية ومقارنة تأثير الاستخدام المزدوج بفعالية استخدام كل من العقاقير لوحده. فُستت الجرذان المختبرية إلى ثمانية مجموعات، كل مجموعة تتكون من خمسة جرذان. المجموعة الأولى لم تجري أي دواء عن طريق الفم لمدة 10 أيام متتالية وحققت تحت الخلب في اليوم الثامن بجرعة 7.5 مل لكل كيلو غرام من وزن الجسم. المجموعة الثانية أيضا لم تجري أي دواء عن طريق الفم لمدة 10 أيام متتالية وحققت تحت الخلب في اليوم الثامن بجرعة مفردة من الأدرياميسين بجرعة 15 ملعم لكل كيلو غرام من وزن الجسم. في المجموعتين الثالثة والسادسة جرعت الجرذان بـ 10 ملغم لكل كيلو غرام من وزن الجسم عن طريق الفم. في المجموعتين الرابع و الثامن، وجرعت الجنين بـ 15 ملعم لكل كيلو غرام من وزن الجسم عن طريق الفم. بعد العلاج بالعقاقير، رزوفاستاتين، و تلمسارين، وجرعت الجرذان بـ 15 ملعم لكل كيلو غرام من وزن الجسم عن طريق الفم. وحققت تحت الخلب في اليوم الثامن بجرعة مفردة من الأدرياميسين بـ 15 ملعم لكل كيلو غرام من وزن الجسم. في اليوم العاشر من الدراسة، سُتقل عينات من الدم من الجرذان للتحليلات المخبرية. لوحظ وجود ارتفاع ملحوظ في مستوى LDH، CTn-I، MDA، IL-17 في المجموعة معالمة بالأدوية. ولاحظ أيضاً حصول انخفاض ملحوظ في مستوى LDH، CTn-I، MDA، IL-17 في المجموعة معالمة بالأدوية. يمكن الاستنتاج أن العقاقير المستخدمة في الدراسة الحالية لها تأثيرات وقائية اتجاه التسمم الحاد للقلب الناتج عن استخدام الأدرياميسين في الجرذان المختبرية.