The Beneficial Effects of Co-administration of Enalapril and Danazol in Rat

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Abstract

Danazol, a weekly androgenic, heterocyclic compound with anabolic properties, is used primarily in the treatment of endometriosis and other gynecological complains. Effects of co-administration of enalapril, angiotensin converting enzyme inhibitor (ACE inhibitor) with danazol on some of biochemical parameters in rats were studied. Treatment of rats with danazol (200mg/kg i.p) for 15 days induced a significant elevation of serum aspartate transaminase activity (AST, EC: 2.6.1.1), triglycerides (TG) and induced a significant decrease in serum cholesterol. However, enalapril treatment (20mg/kg i.p) for 15 days caused a significant elevation of serum alanine transaminase activity (ALT, EC: 2.6.1.2).

Co-administration of danazol and enalapril significantly lower the elevated level of serum transaminases enzyme activities and serum TG. It could be concluded that long term treatment with enalapril might enhance the use of danazol in clinical situation through protection against undesirable side effects.
Introduction

Danazol is a heterocyclic compound used in the treatment of endometriosis and other gynecological complains. It has a weakly androgenic with an anabolic properties\(^1\). Its inhibitory action on gonadotropin-releasing hormone and gonadotropin secretion results in suppression of menstruation, inhibition of ovulation and endometrial atrophy\(^2\). The hypothalamic-pituitary effects of danazol are specifically directed at gonadotropin synthesis or release and it seems to have no action on the production of other pituitary hormones e.g. adrenocorticotropin\(^2\). However, Tamura et al\(^3\) have reported that danazol inhibitory actions on ovulation and ovarian prostaglandin F\(_2\) alpha metabolism may occur via some direct effects on the ovary. This was confirmed by Kogo et al\(^4\) who clearly demonstrate that direct action of danazol on the ovary and uterus may contribute to its therapeutic effects. Therapy with danazol produces a rapid reduction in high-density lipoprotein (HDL) cholesterol coupled with a rise in the pro-atherogenic low-density lipoprotein (LDL). These apparently unwanted actions are balanced by a possibly beneficial reduction in the atherogenic lipoprotein (a) fraction\(^5\).

It has been found that total lipids increased in the rat ovaries with danazol treatment, triglycerides, the stored form of lipids constitute the major components of lipids in the treated ovaries. However, the amount of phospholipids, glycolipids, cholesterol and free fatty acids decreased in the ovaries with increased danazol treatment\(^6\). Chronic administration of danazol inhibited RNA synthesis, protein and sialic acid and increased total cholesterol in testes\(^7\). However, this effect was reversible after 60 days of cessation of drug administration.

The mechanism for these changes induced by danazol on lipid profile is still unknown, but probably relates to the effect of danazol on hepatic lipase, LDL receptor
and lecithin-cholesterol acyltransferase (LCAT) activity. In addition, unwanted endocrine effects have been reported, including mild deterioration in glucose tolerance increased insulin resistance, level of sex hormones and thyroxine binding globulins.

In certain cases of danazol therapy, concomitant administration of enalapril, angiotensin-converting enzyme inhibitor (ACE inhibitor), to manage cardiovascular events as hypertension, arrhythmia or congestive heart failure. In such circumstances, it is essential to rule out any drug interaction, which might disrupt the control of ACE inhibitor on cardiovascular events. No previous study have been investigated the potential for these interaction between both drugs.

The present study was attempted to examine this issue by studying the potential changes in the biochemical parameters with danazol administration alone or in combination with enalapril.

**Material and Methods**

**Animals**

Male albino rats, 8-10 weeks old, weighing 150-200 g. they were obtained from the Experimental Animal Care Center of King Saud University, Riyadh, KSA. Animals were maintained under standard conditions of humidity with regular light/dark cycle and free access to food (Purina Chow) and water. The animals were housed in-groups to acclimatize to the laboratory conditions.

**Drugs**

Danazol, steroidal isoxazole, (Winthrop, New York, USA), Enalapril (Merk Sharp, Dohme B.V. Haarlem-Netherland) were used. All the other reagents and chemicals used in this work were of analytical reagent grade.
The drug solutions were prepared freshly daily. Enalapril was dissolved in normal saline 0.9%, while danazol was suspended in 0.5% carboxymethyl cellulose.

The dose of enalapril selected was 20mg/kg, while danazol was 200 mg/kg.

**Protocols and administration procedures**

Twenty rats were randomly divided into four groups. These groups received the following treatments:-

Group 1: served as control group; group 2 was injected intraperitoneally (i.p.) with enalapril 20mg/kg; group 3 was injected with danazol 200 mg/kg while group 4 was injected enalapril 20 mg/kg and danazol 200 mg/kg for 15 days.

The rats were fasted for twenty hours after last injection. The rats were anaesthetized using diethylether. Blood was obtained from animal and immediately centrifuged, plasma was isolated and kept at -20˚C until analysis for biochemical parameters.

**Determination of plasma enzyme activities**

Plasma alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were determined according to Reitman and Frankel\(^\text{10}\) and Fujita\(^\text{11}\). Creatine phosphokinase (CPK) was determined kinetically as described earlier by Swannson and Wilkinson\(^\text{12}\).

**Determination of plasma biochemical parameters**

Plasma level of cholesterol and triglycerides were determined according to Trinder\(^\text{13}\) while plasma level of urea and creatinine were estimated according to Hallet and Cook\(^\text{14}\) and Bonsness and Taussky\(^\text{15}\).
Statistics
Values are given as means±SEM. The level of statistical significance was taken at p<0.05 using one-way ANOVA followed by Tukey and Kramer multiple comparison test to judge the difference between various groups.

Results

Effects of danazol, enalapril and co-administration on the plasma enzyme activities
Administration of danazol 200mg/kg for 15 days lead to significant increase in AST enzyme activity (246.2±34.2 M±SE), while 20 mg/kg enalapril administration for 15 days significantly increase ALT enzyme activity (27.4±0.4 M±SE). However, co-administration of danazol and enalapril normalize the elevated levels of plasma ALT and AST (Table 1). These results indicated that the combination of both danazol and enalapril had a beneficial effects against side effects induced by administration of danazol or enalapril alone (Tab 1). Danazol or enalapril or both can not alter the level of ALP and CPK.

Effects of danazol, enalapril and their combination on the plasma biochemical parameters
Administration of danazol for 15 days (200mg/kg) caused a significant elevation of plasma triglycerides (159±7mg% Mean±SE) (P<0.01). In contrast plasma cholesterol was significantly decreased. Treatment with enalapril did not alter plasma level of triglycerides or cholesterol. Co-administration of danazol and enalapril significantly lower plasma triglycerides level 74±7.4 mg% Mean+SE), and the cholesterol level
was still significantly lower. Plasma level of creatinine and urea can not altered by administration of danazol, enalapril or both (Tab 2).

**Discussion**

To clarify the effect of co-administration of danazol with enalapril, the present study focused on the changes in some of the biochemical parameters. Early reports indicated that danazol treatment had little effect on plasma lipid (TG and cholesterol) levels\textsuperscript{16,17}, but recently, it has been reported that danazol produces a rapid reduction in high density lipoprotein (HDL) cholesterol, particularly cardioprotective HDL\textsubscript{2} subfraction, coupled with a rise in the proatherogenic low density lipoprotein (LDL)\textsuperscript{1}.

Sangha and Chopra\textsuperscript{6} demonstrated that danazol significantly increased total lipid in the treated ovaries. However, the amount of phospholipids, glycolipids, cholesterol and free fatty acids decreased in the ovaries with increased danazol treatment.

The results of the present study indicate the possibility that enalapril can protect against hypertriglyceridemia induced by administration of danazol in male swiss albino rats. Treatment of rats with enalapril (20 mg/kg i.p) for 15 days neither induced any changes in the biochemical parameters measured. However, ALT significantly increased. The co-administrations of both danazol and enalapril have a beneficial effect. This was evidenced by a significant reduction of serum transaminases enzyme activities induced by administration of both drugs and reduction of plasma TG level induced by danazol.

The data of the present study confirm the results of the previous study that danazol therapy induced a significant increase in the plasma TG level. However, it has been disputed by Henzl et al\textsuperscript{18} who reported that LDL cholesterol rise significantly during danazol treatment. The effect of danazol therapy on LDL cholesterol was variable and not as consistent feature as HDL cholesterol reduction\textsuperscript{19}, the most consistent lipid
related changes, which follows danazol therapy. The greater reduction was in HDL$_2$ and HDL$_3$$^{20,21}$. Valimaki et al$^{22}$ reported that danazol administration had a little effect or no impact on plasma cholesterol or TG or on the size and composition of VLDL in the circulation and concluded that danazol effect on coronary risk not due to its effect on total lipid but due to influence on the dynamics of lipoprotein metabolism.

The mechanism whereby enalapril might protect against the undesirable side effect of danazol is not well understood at this time. It has been demonstrated that danazol decreased plasma fibrinogen, lipoprotein (a) levels, promotes fibrinolysis and cause a rise in plasminogen$^2$. Such changes should be considered beneficial leading to inhibition of process of thrombosis. However, it is difficult to weigh the increased potential for lipid deposition against reduction in clotting tendency in the atherosclerotic formation. In the other hand, enalapril is an ACE inhibitor and the beneficial effects of this class of drug have been related to a decreased in angiotensin II$^{23}$. However, ACE inhibitors possess several other modes of actions, including inhibition of bradykinin$^{23}$, as its enzymatic degradation can be thought by ACE (kininase II). Enalapril may be potentiate the beneficial effects of danazol and thus decreased plasma lipids through improvement of lipoprotein metabolism.

The usual recipients of danazol therapy are pre-menopausal females in whom the absolute risk of ischemic heart decrease (IHD) is low. However, in the presence of other IHD risk factors (smoking, hypertension, hyperlipidemia and IHD family history), enalapril can be considered as an alternative therapy to control plasma lipoprotein changes.

In conclusion: Co-administration of enalapril-danazol therapy results in an unexpectly suppression of serum transaminases activities and TG. This may enhance the use of danazol in clinical situation.
References

11. Fujjta H (1939) J. Biochem (Japan) 30:69-87


**Table 1:** Effect of enalapril, danazol and both on plasma enzyme activities

<table>
<thead>
<tr>
<th></th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>CPK (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.9 ± 0.9</td>
<td>134 ± 9.5</td>
<td>185±45.4</td>
<td>206±48.8</td>
</tr>
<tr>
<td>Danazol</td>
<td>22.4±0.6</td>
<td>246±34.2*</td>
<td>148.2 ± 30.1</td>
<td>445 ±74.8</td>
</tr>
<tr>
<td>Enalapril</td>
<td>27.4±0.4*</td>
<td>172.6 ±44.06</td>
<td>169.5±36.5</td>
<td>338±96.1</td>
</tr>
<tr>
<td>Danazol + Enalapril</td>
<td>23.8±1.4</td>
<td>107 ±16.05</td>
<td>109±4.2</td>
<td>278±34.3</td>
</tr>
</tbody>
</table>

Five animals were used in each group. * Significance from control group P<0.05
Table 1: Effect of enalapril, danazol and both on plasma biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>TG Mg%</th>
<th>Colesterol Mg%</th>
<th>Urea Mg%</th>
<th>Creatinine Mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>113.8 ± 11.3</td>
<td>86 ± 7.4</td>
<td>73 ± 8.5</td>
<td>0.7 ± 0.06</td>
</tr>
<tr>
<td>Danazol</td>
<td>159 ± 7**</td>
<td>53 ± 2.1*</td>
<td>71.6 ± 9.7</td>
<td>0.69 ± 0.08</td>
</tr>
<tr>
<td>Enalapril</td>
<td>135 ± 6.6</td>
<td>88 ± 7.6</td>
<td>92 ± 5.3</td>
<td>0.6 ± 0.02</td>
</tr>
<tr>
<td>Danazol + Enalapril</td>
<td>74 ± 7.4*</td>
<td>49 ± 2.6**</td>
<td>74 ± 9.1</td>
<td>0.59 ± 0.06</td>
</tr>
</tbody>
</table>

Five animals were used in each group. * Significance from control group P<0.05