

The Beneficial Interaction Between Enalapril and Danazol in Normal 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 complaints. Effects of co-administration of enalapril, an angiotensin converting enzyme inhibitor (ACE inhibitor) with danazol on some 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 lowered the elevated levels of serum transaminases enzyme activities and serum TG. It could be concluded that long term treatment with enalapril might be beneficial in patients danazol in clinical situations through protection against undesirable side effects.

Introduction

Danazol is a heterocyclic compound used in the treatment of endometriosis and other gynecological complaints. It is a weakly androgenic compound with an anabolic properties¹. Its inhibitory action on gonadotropin-releasing hormone and gonadotropin secretion results in suppression of menstruation, inhibition of ovulation and endometrial atrophy². 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. adrenocorticotropin². However, Tamura et al³ have reported that danazol inhibitory actions on ovulation and ovarian prostaglandin F₂ alpha metabolism may occur via some direct effects on the ovary. This was confirmed by Kogo et al⁴ who clearly demonstrated that the 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) fraction⁵.

It has been found that total lipids are increased in the rat ovaries with danazol treatment. Triglycerides, the stored form of lipids, constitutes 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 treatment⁶. In addition, chronic administration of danazol inhibited RNA synthesis, protein, sialic acid synthesis and increased total cholesterol in testes⁷. However, these effects were 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 related to the effect of danazol on hepatic lipase activity, LDL

receptors and lecithin-cholesterol acyltransferase (LCAT) activity¹. Moreover, additional unwanted endocrine effects have been reported, including mild deterioration in glucose tolerance, increased insulin resistance, decreased level of sex hormones and thyroxine binding globulins⁸.

In certain cases of danazol therapy, concomitant administration of enalapril, an angiotensin-converting enzyme inhibitor (ACE inhibitor), to manage the cardiovascular events as hypertension, arrhythmia or congestive heart failure⁹. In such circumstances, it is essential to rule out any drug-drug interaction, which might disrupt the control of ACE inhibitor on cardiovascular events. No previous study have been performed to investigate the interaction between these drugs. Therefore, the present study was attempted to examine this issue by studying the changes in some biochemical parameters that may result from danazol administration alone or in combination with enalapril.

Material and Methods

Animals

Male albino rats, 8-10 weeks old, weighing 150-200 g, 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, was obtained from (Winthrop, New York, USA). Enalapril was a product of (Merck Sharp and Dohme B.V. Haarlem-Netherland). All remaining chemicals were of the highest grade commercially available

The drug solutions were prepared freshly daily. Enalapril was dissolved in normal saline 0.9%, whereas danazol was suspended in 0.5% carboxymethyl cellulose.

Based on our own preliminary experiments, the dose of enalapril and danazol were selected as 20mg/kg and 200mg/kg respectively.

Protocols and administration procedures

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

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

The rats were fasted for twenty hours after last injection. They were anaesthetized using diethylether. Blood samples were obtained from each animal and immediately centrifuged, the 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) were determined according to Reitman and Frankel¹⁰ while alkaline phosphatase (ALP) was determined according to Fujita¹¹ Creatine phosphokinase (CPK) was determined kinetically as described earlier by Swanson and Wilkinson¹².

Determination of plasma biochemical parameters

Plasma levels of cholesterol and triglycerides were determined according to Trinder¹³ while plasma levels of urea and creatinine were estimated according to Hallet and Cook¹⁴ and Bonsness and Taussky¹⁵

Statistics

Values are given as means \pm SEM. The level of statistical significance was taken at $p=0.05$ using one-way ANOVA followed by Tukey Kramer multiple comparison test to judge the difference between various groups.

Results and 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. Administration of danazol 200mg/kg i.p for 15 days lead to significant increase in AST enzyme activity (246.2 \pm 34.2 M \pm SE), while 20 mg/kg i.p enalapril administration for 15 days significantly increased ALT enzyme activity (27.4 \pm 0.4 M \pm SE). Co-administration of danazol and enalapril normalized the elevated levels of plasma ALT and AST (Table 1). These results indicated that the combination of both danazol and enalapril had a beneficial effect against some of the undesirable side effects induced by administration of danazol or enalapril alone. Treatment with danazol, enalapril or both did not alter ALP and CPK enzymes activities.

Administration of danazol (200mg/kg i.p) for 15 days caused a significant elevation of plasma triglycerides (159 \pm 7 M \pm SE) ($P<0.01$). In contrast plasma cholesterol was significantly decreased. Treatment with enalapril did not alter plasma levels of triglycerides or cholesterol. Co-administration of enalapril with danazol significantly lowered plasma triglycerides level (74 \pm 7.4 M \pm SE) while the cholesterol level was still significantly lower. Plasma levels of creatinine and urea were not altered by the administration of danazol, enalapril or both (Table 2).

Early reports indicated that danazol treatment had little effect on plasma lipids (TG and cholesterol) levels^{16,17}, but recently, it has been reported that danazol produces a

Table 1 : Effect of enalapril, danazol and their combination on plasma enzyme activities

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

All data represent mean values ± SE (n=5).

Danazol (200mg/kg i.p), enalapril (20mg/kg i.p) and both were given for 15 consecutive days. Blood samples were obtained and plasma were isolated

* Significant difference from control group.

* P < 0.05

Table 2 : Effect of enalapril, danazol and their combination on plasma biochemical parameters

	TG mg/dl	Cholesterol mg/dl	Urea mg/dl	Creatinine mg/dl
Control	113.8 ± 11.3	86 ± 7.4	73±8.5	0.7±0.06
Danazol	159±7**	53 ±2.1*	71.6 ± 9.7	0.69 ±0.08
Enalapril	135±6.6	88±7.6	92±5.3	0.6±0.02
Danazol + Enalapril	74±7.4*	49 ±2.6**	74±9.1	0.59±0.06

All data represent mean values ± SE (n=5).

Danazol (200mg/kg i.p), enalapril (20mg/kg i.p) and both were given for 15 consecutive days. Blood samples were obtained and plasma were isolated

* Significant difference from control group.

* P < 0.05

rapid reduction in high density lipoprotein (HDL) cholesterol, particularly cardioprotective HDL₂ subfraction, coupled with a rise in the proatherogenic low density lipoprotein (LDL) ¹. Sangha and Chopra⁶ demonstrated that danazol significantly increased total lipids 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 are compatible with the data of the previous study demonstrating that danazol therapy induced a significant increase in the plasma TG level. However, our study showed that danazol treatment caused a significant decrease in plasma total cholesterol. These results are not in agreement with Henzl et al¹⁸ 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 reduction¹⁹, the most consistent lipid related changes, which follow danazol therapy. The greater reduction was in HDL₂ and HDL₃ ^{20,21}. Valimaki et al²² reported that danazol administration had a little effect on plasma cholesterol or on the size and composition of VLDL in the circulation and concluded that danazol effect on coronary risk is not due to its effect on total lipid but it may be due to influence on the dynamics of lipoprotein metabolism.

Treatment of rats with enalapril (20 mg/kg i.p) for 15 days did not induce any changes in the biochemical parameters measured. However, ALT activity was significantly increased. The results of the present study indicate the possibility that enalapril can protect against hypertriglyceridemia induced by administration of danazol in normal rats. The co-administration of enalapril and danazol have a beneficial effect. This was evidenced by a significant reduction of serum

transaminases enzyme activities induced by administration of either drug alone. In addition increased plasma TG level induced by danazol was significantly reduced.

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 plasminogen². Such changes should be considered beneficial leading to inhibition of the process of thrombosis. However, it is difficult to assess that the increased potential for lipid deposition against reduction in clotting tendency in the atherosclerotic formation. On the other hand, enalapril is an ACE inhibitor and the beneficial effects of this class of drug have been related to a decrease in angiotensin II²³. However, ACE inhibitors possess several other modes of actions, including inhibition of bradykinin²³, as its enzymatic degradation can be thought by ACE (kininase II). Enalapril may potentiate the beneficial effects of danazol and thus decreases 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 diseases (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: Combined enalapril-danazol therapy results in an unexpectedly suppression of serum transaminases activities and TG, which is valuable in some clinical situations.

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