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Hemorheological Effects of Long-Term Administration of Combined Oral Contraceptive in Rats

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Abstract: This study examined the effect of administration of combined Oral Contraceptive (OC) on blood viscosity and associated hemorheological parameters such as plasma viscosity, Pack Cell Volume (PCV), serum albumin, fibrinogen and total plasma proteins. Female wistar rats aged 7-10 weeks were selected and randomly distributed into two groups, the control group and the OC-treated group, with 20 rats in each group. The rats in both groups were fed with standard rat chow. After two weeks of acclimatization, rats in OC-treated group received OC therapy (containing 1.0 µg ethinyloestradiol and 10.0 µg of norgestrel), while rats in the control group remained on standard rat chow. After 7 weeks of treatment, hemorheological parameters were determined using standard hemorheological techniques described by Dacies and Lewis. Unpaired t-test was performed in all data with the significant level set at $p < 0.05$. There was no significant change in level of fibrinogen, while there was significant increase in blood viscosity, plasma viscosity, PCV, serum albumin concentration and total plasma proteins in OC-treated rats. This finding suggests that increase blood viscosity and plasma viscosity seen in OC therapy is associated with an increase in PCV and serum albumin level.

Key words: Oral contraceptive, blood viscosity, plasma viscosity, plasma proteins

INTRODUCTION

Several hemorheological and haemostatic parameters has been associated closely with hormone replacement therapy. Such parameters include blood viscosity, plasma viscosity, haematocrit value, fibrinogen level, fibrinolytic activity and thromboxane level (Moreau *et al.*, 2003). Studies have shown that selective use of hormone therapy may alter these relationships with ageing (Moreau *et al.*, 2003). Hormone therapy has also been shown to influence blood flow (Dinno *et al.*, 1999, 2001; Dunbar and Kenney, 2000; Moreau *et al.*, 2003).

Hormone substitution has been shown to benefit plasma viscosity (Persico *et al.*, 2004; Mancini *et al.*, 2005), thereby improving blood flow. It has also been shown that blood flow declines in estrogen-deficient women due to reduction in vascular resistance and increase in estrogen-supplemented therapy (Moreau *et al.*, 2003). Studies have shown that promotion of blood flow is caused mainly by decreased vascular resistance (Shaw *et al.*, 1984; Chapman *et al.*, 1997; Speroff *et al.*, 1999). Decrease estrogen level in healthy women associated with age has been demonstrated to cause reduced arterial blood flow (Dinno *et al.*, 2001).

Plasma viscosity has also been shown to decrease in estrogen therapy in postmenopausal women (Mancini *et al.*, 2005). Conversely, previous studies document that the increase in the concentration of estrogen in follicular phase has no effect on blood viscosity and haematocrit value (Krejza *et al.*, 2000).

On the other hand, administration of Oral Contraceptive (OC) steroids has been shown to increase plasma viscosity and haematocrit value (Lowe *et al.*, 1980). Studies have also shown that low dose OC leads to no biologically meaningful changes in blood rheology, while higher than normal dose induce limitation of blood fluidity (Ernst *et al.*, 1989).

The use of extremely high-dose OC is associated with venous thromboembolism, myocardial infarction, stroke, hypertension and breast cancer (Farmer and Preston, 1995). The risk of older users, who smoke is substantial (Sidney *et al.*, 1996). However, little or no reports have been documented on the influence of combined OC therapy on hemorheological parameters.

The aim of this study is to investigate the effect of combined OC therapy on blood viscosity and hemorheological parameters associated with blood viscosity.

MATERIALS AND METHODS

Experimental animals: The experiment was conducted in the animal house of the University of Ilorin, Ilorin, Kwara state, Nigeria.

Experiments were conducted in 40 wistar rats aged 7-10 weeks. They were randomly distributed into two equal groups, housed in a wired-bottomed, stainless steel cages in a well ventilated, temperature-controlled room at 25°C on 12:12 light-dark cycle.

Treatments: Both groups fed on standard rats chow for 2 weeks after which the OC-treated group received OC containing 1.0 µg ethinyloestradiol and 10.0 µg norgestrel (Wyeth Ayerst Inc., Canada) intragastrically for 7 weeks, while the control group remained on the standard rat chow throughout the 7 weeks of experiments. OC-treated rats received OC therapy early in the morning between 07:00 and 08:00 h. All experimental procedures conformed to Guiding Principle for Research Involving Animals.

Measurements: Blood samples were collected weekly to determine blood viscosity throughout the 2 weeks of the acclimatization period and 7 weeks of treatment. After the 7th week of treatment, the rats were sacrificed and blood was collected through jugular (cervical) puncture to determine hemorheological parameters.

Blood viscosity was determined using the Ostwald viscometer. Relative blood viscosity was determined by comparing the blood viscosity with water viscosity. Pack cell volume was determined by centrifuging the whole blood for about 30 min at the speed of 3000 rpm (revolutions per minute). Total plasma proteins, serum albumin and fibrinogen levels were assayed using a standard kit (Randox).

Statistical analysis: Statistical analyses were performed with SPSS windows version 10.0. Unpaired t-test was performed in all data with the significant level set at $p < 0.05$. Data are presented as Mean±SEM.

RESULTS

Following OC administration, blood viscosity of the OC-treated group increased significantly throughout the seven weeks of treatment.

Figure 1 shows the significant increase of blood viscosity that occurred in the OC-treated group with the change in blood viscosity reaching 4.421 ± 0.4 from the initial 3.218 ± 0.7 . The change in blood viscosity of the control group was not significant. Changes in blood viscosity is seen to be associated with OC administration.

PCV level was significantly increased in OC-treated group when compared with the control group at the end of the 7th week of the therapy (13%) (Fig. 2).

Plasma viscosity was significantly increased in OC-treated group following the administration of OC. This is associated with the significant increase in blood viscosity. However, there were little changes in the Relative Blood Viscosity (RBV) and Relative Plasma Viscosity (RPV) in the OC-treated group when compared with the control group (Table 1).

Serum albumin level was significantly increased in the OC-treated group when compared with the control group ($p < 0.05$). Similarly, total plasma proteins was significantly increased in the OC-treated group when compared with the control group ($p < 0.05$). However, there was no significant difference in the fibrinogen level in both groups (Table 2).

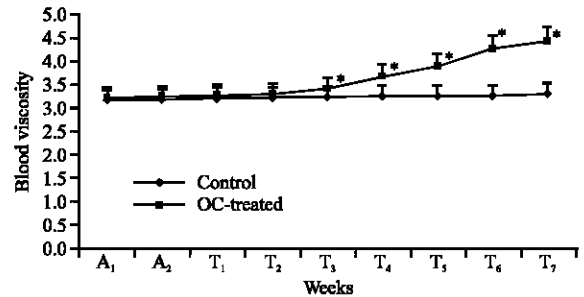


Fig. 1: Changes in blood viscosity in control and OC-treated groups during the acclimatization period (A₁ and A₂) and treatment period (T₁ to T₇), * $p < 0.05$ versus control

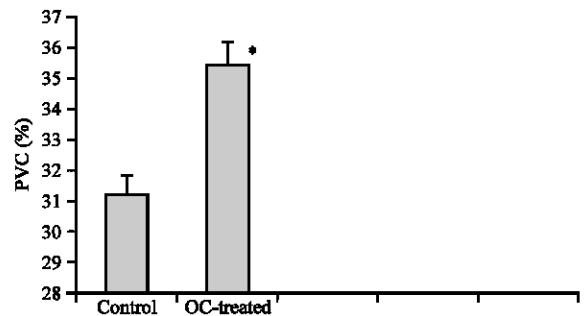


Fig. 2: Effect of oral contraceptive administration on Pack Cell Volume, * $p < 0.05$ versus control

Variables	Control	OC-treated
Blood viscosity	3.310±0.60	4.421±0.4*
Relative blood viscosity	3.980±0.22	4.320±0.21
Plasma viscosity	1.361±0.89	1.468±0.71*
Relative plasma viscosity	1.800±0.20	2.010±0.19

Data are shown as Mean±SEM, * $p < 0.05$ versus control

Table 2: Effect of oral contraceptive on some blood viscosity determinants

Variables	Control	OC-treated
Serum albumin (g %)	4.10±0.2	5.30±0.1*
Fibrinogen (g %)	0.22±0.1	0.28±0.2
Total plasma proteins (g %)	6.90±0.4	8.20±0.3*

Data are shown as Mean±SEM, *p<0.05 versus control

DISCUSSION

A well-known consequence of most common hormone replacement therapy is an increase in plasma triglycerides that occurs through estrogen-induced stimulation of hepatic VLDL secretion and inhibition of hepatic triglyceride lipase (Applebaum-Bowden *et al.*, 1989). The increase in plasma triglycerides has been shown to be associated with elevations in plasma viscosity (Rosenson *et al.*, 1996). Studies have also documented that OC users had no significant changes in plasma viscosity, but these conclusions were limited by small sample size (Drummond *et al.*, 1980). A study of women treated with triphasic OC showed an increase in plasma viscosity after 3-6 months of treatment (Coata *et al.*, 1995). Some studies have shown that estrogen replacement therapy lowers plasma viscosity (Mancini *et al.*, 2005; Persico *et al.*, 2004). However, none of these studies has shown the weekly changes in blood viscosity during OC therapy. The novel findings of this present study document the weekly changes in blood viscosity in relation to some blood viscosity determinants.

The present study shows that there was a steady increase in blood viscosity after the acclimatization period, during OC therapy. There was no significant difference in the increase in blood viscosity between both groups until the 5th week of experiment when the difference raised by 5%, after which the difference raised steadily throughout the weeks of experiment. Prior to OC therapy, there was no significant difference in blood viscosity in both groups (Fig. 1).

Present findings that combined OC therapy increases blood viscosity are consistent with previous studies of Ernst *et al.* (1989), Applebaum-Bowden *et al.* (1989) and Rosenson *et al.* (1996). Although, this is not in agreement with some previous studies (Dinno *et al.*, 1999, 2001). This could suggest that the progesterone component of the OC may be responsible for the raise in the blood viscosity. However, this study shows that there was little difference in the RBV and RPV in both groups (Table 1).

This study also documents the effect of OC therapy on PCV. This study to our observation is the first study to document the effect of OC administration on PCV. The observations in this study show that there was significant difference in PCV (p<0.05). PCV increased significantly in

OC-treated group by 13% when compared with the control group (Fig. 2). This is in agreement with previous studies of Lowe *et al.* (1980). The raise in PCV could suggest an increase in the production of red blood corpuscles, which is a key determinant of blood viscosity and plasma viscosity. This finding provides evidence that the raise in blood viscosity and plasma viscosity seen in OC-treated rats is likely to be a secondary effect to the erythropoietic effect of OC steroids.

Present findings also show that OC-treatment had significant effects on plasma proteins. After 7 weeks of OC therapy, there was significant raise in serum albumin level (29%) in the OC-treated rats. A similar raise was seen in total plasma proteins level (19%). However, there was no significant change in fibrinogen level. This is in consonance with previous studies (Rosenson, 1993; Rosenson *et al.*, 1994, 1996, 1998; Lowe *et al.*, 1980; Mancini *et al.*, 2005). This finding shows that the increase in blood viscosity is associated with the raise in serum albumin level and total plasma proteins. This could suggest that combined OC steroids have an effect on the hepatic synthesis of plasma proteins, particularly, serum albumin.

This is the first report that evaluates the hemorheological effects of long-term administration of combined OC in rats and documents the effects of OC steroids on PCV, RBV, RPV, total plasma proteins, serum albumin and fibrinogen. Present study shows a new mechanism through which OC therapy increases blood viscosity and plasma viscosity. Further studies are needed to evaluate factors that can improve blood viscosity and plasma viscosity, which is associated with a significant raise in PCV and serum albumin.

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