Original Article

Risk of Venous Thromboembolism from Use of Dienogest: Experimental Study on Rats

Hadeel Anwer Alsarraje and Liqaa Khalel Alhyali

College of Medicine, University of Mosul, Mosul, Iraq

ABSTRACT

Background and objective: We aimed to identify the effects of dienogest on inducing thromboembolism in rats following exposure to dienogest for a period of either 10 days (2 estrus cycles), 20 days (4 estrous cycles), or 30 days (6 estrus cycles).

Materials and Methods: To do so, 40 rats were divided into 4 groups; dienogest-free control group, dienogest treated for 10 days (G1), 20 days (G2), and 30 days (G3). Then lungs and femoral veins were collected from subjects after sacrificing them, and these tissues were fixed for histological analysis.

Results: Results showed that venous thromboembolism increased with dienogest therapy as indicated by the increased score of inflammation in the lung tissues, alongside increased thickness of femoral vein wall, and histological findings of fibrin deposition, vessel congestion, inflammatory cells infiltrations, and epithelial desquamation.

Conclusion: To sum up, dienogest long-term therapy could be a risk for cardiac thrombotic diseases, and therefore we do advise using alternative progesterone or adding estrogen in low doses to minimize toxic effects primarily when used as postmenopausal replacement therapy.

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Key Words: Contraceptives, dienogest, femoral vein, lung, thrombosis.

Corresponding Author: Hadeel Anwer Alsarraje, PhD, College of Medicine, University of Mosul, Mosul, Iraq, **Tel.**: +964 7705 2619 20, **E-mail:** hadeel.anwar@uomosul.edu.iq

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INTRODUCTION

Combined oral contraceptive pills (COCs) are still a common form of birth control[1,2]. Since general practitioners frequently interact with women regarding contraception[1], they are required to give patients substantial proof information regarding the advantages and disadvantages of all available contraceptive methods. Healthcare providers must balance emotive disinformation and misconceptions regarding the dangers of COCs, particularly the risk of venous thromboembolism (VTE), with fair, simple-to-understand facts in light of the rise of social media as a source of medical information. Combined oral contraceptives fall into four generations listed in (Table 1), dienogest is a member of the fourth generation of oral contraceptive medications.

Table 1: Generation of combined oral contraceptive medications[1]

No.	First Generation	Second Generation	Third Generation	Fourth Generation
1	Norethynodrel	Microgynon (Ethinylestradiol /Levonorgestrel)	Cilest (Ethinylestradiol/ Norgestimate)	Yasmin (ethinylestradiol and drospirenone).
2	Lynestrenol	Logynon (Ethinylestradiol/ Levonorgestrel)	Marvelon(Ethinylestradiol/ Desogestrel)	Zoely (estradiol hemihydrate and nomegestrol acetate)
3	Ethynodiol diacetate	Loestrin (Ethinylestradiol/ Norethisterone)	NuvaRing (Ethinylestradiol/ Etonogestrel)	Qlaira (estradiol valerate and dienogest).
4	-----	-----	EvraPatch(Ethinylestradiol /Norelgestromin)	-----

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Although venous thromboembolism (VTE) is uncommon in healthy, childbearing women (incidence 5–10 occurrences per 10,000 women-years), the use of combined oral contraceptives (COC) can raise the risk of VTE, including deep venous thrombosis and pulmonary embolism, in comparison to nonuse[3,4]. However, the prevalence of VTE amongst COC consumers is still low (8–10 occurrences per 10,000 women-years of consumption), and substantially reduce the prevalence of VTE during pregnancy and the postpartum period[5,6]. Historically, it was believed that the impacts of COCs on the risk of thrombosis were primarily due to the actions of estrogen on hemostatic markers. Nevertheless, research has shown that the risk of VTE differs amongst women who use COCs that include various progestogens.

In spite of the fact that the particular formulation of 2 mg of dienogest (DNG) and 30 g of ethinylestradiol (EE) enjoys a sizable share in the market in Europe, there is little published evidence on VTE risk. The risk of VTE involved in a specific combination (DNG/EE) compared to COCs incorporating levonorgestrel (LNG)/EE, which is frequently cited as the reference standard for the VTE risk related to combined hormonal contraceptives, is presently unclear experimentally[7].

Drospirenone-containing oral contraceptive has been reported to induce thromboembolism compared to other COCs[8]. Estradiol-containing contraceptive is associated with venous thromboembolism[9]. The incidence of VTE is reported to be low in progesterone-only pills[10-13]. The risk of VTE is increased with the addition of estrogen even at low doses[14] with few studies confirming that the newer generation is associated with a greater risk of VTE than the first and second generation[15-18]. In the present study, we aimed to identify the thrombogenic effects of dienogest on rat models.

MATERIAL AND METHODS

A total of 40 rats (12-14 weeks old White Norway Albino adult female; weight 165-250 grams). The animals were housed in Veterinary Collage in Laboratory Animal House. They were maintained at room temperature (23 2 degrees C) with a 12 hr light / 12 hr darkness cycle. Rats were placed in specially designed plastic cages, the floor of cages was covered with sawdust, free of materials against the ground insect, and was replaced weekly. The rats were allowed to feed on a standard commercial diet and tap water to the point of satiety. The animals were adopted and allocated into 4 groups, each group had 6 rats; a control group, and three other groups that were orally given a daily dose of 2 mg dienogest (0.3 mg/kg/day to rats) for periods of 10 days (2 estrus cycles for female rats which correlate to tow menstrual period for a reproductive adult female in human), 20 days (4 estrus cycles for female rats which correlate to four menstrual periods for a reproductive adult female in human), and 30 days (6 estrus cycles for female rats which correlate to six menstrual periods for a reproductive adult female in human). At the end of each period, rats were anaesthetized by ether before sacrifice, and their lung was dissected for histopathological studies and diagnosis of venous thrombosis[19].

The lung and femoral vein tissues were fixed in formalin overnight. Slices (10 microns) of tissue were prepared, paraffinized, and stained with eosin and hematoxylin.

RESULTS

The histological results of rats' lungs of the control group (without treatment) showed normal architectures of lung tissue alveoli, respiratory bronchiole and blood vessels. The lungs of the treated group G1 (10 days) showed mild thickening of alveolar septa by inflammatory cells, thickening of the wall of the bronchiole with inflammatory cells infiltration, necrosis of epithelial cells lining the bronchioles, and hyperplasia of blood vessels. The lung of treated group G2 (20 days) showed interstitial pneumonia represented by thickening of the alveolar septa by inflammatory exudate and haemorrhage, thickening of the wall of the bronchioles with inflammatory cells infiltration, necrosis, and desquamation of epithelial cells lining the bronchioles and emphysema. The rat's lung of the treated group G3 (30 days) shows interstitial pneumonia represented by thickening of alveolar septa by severe inflammatory cells infiltration, thickening of the wall of the bronchioles with inflammatory cells, necrosis and desquamation of epithelial cells lining bronchioles and emphysema (Figure 1). The inflammation score of the non-treated control group was close to 0, while after dienogest therapy score was increased (G1=1.5, G2=2, and G3=2.5), see (Figure 1).

The histological results of rats' femoral veins of the control group (without treatment) showed normal architecture of tunica intima, tunica media, and tunica adventitia. The rats' femoral vein of the treated group G1 (10 days) showed severe congestion in the lumen, thickening of the tunica media and tunica adventitia. The rats' femoral vein of the treated group G2 (20 days) showed recent thrombus (represented by fibrin, white blood cells, and red blood cells) and congestion. The rats' femoral vein of the treated group G3 (30 days) showed the presence of a recent thrombus (represented by fibrin, white blood cells and red blood cells) and thickening of vein layers (figure 2). The thickness of the femoral vein increased with the duration of dienogest treatment. It was 30µm on average in the control group and increased with dienogest therapy (G1=40µ, G2=70µ, and G3=100µm), see (Figure 2).

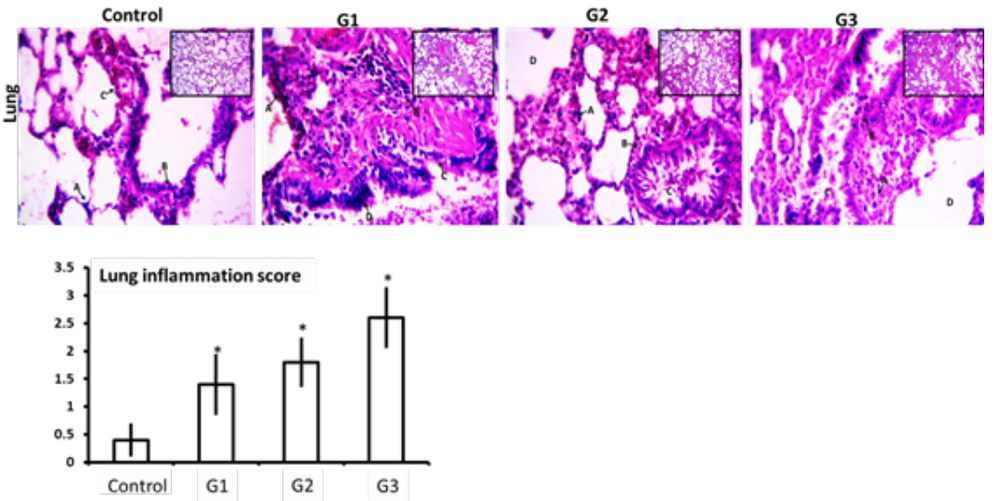

Fig. 1: A representative image for rats lung tissues in the control group versus dienogest treated groups over three-time points (after 10 days exposure (G1), after 20 days exposure (G2), and after 30 days exposure) with lung inflammation scores increasing after exposure to dienogest. Small box 100µm and large 400µm. Eosin-hematoxylin stain.Small inserted images 100X, large images 400X

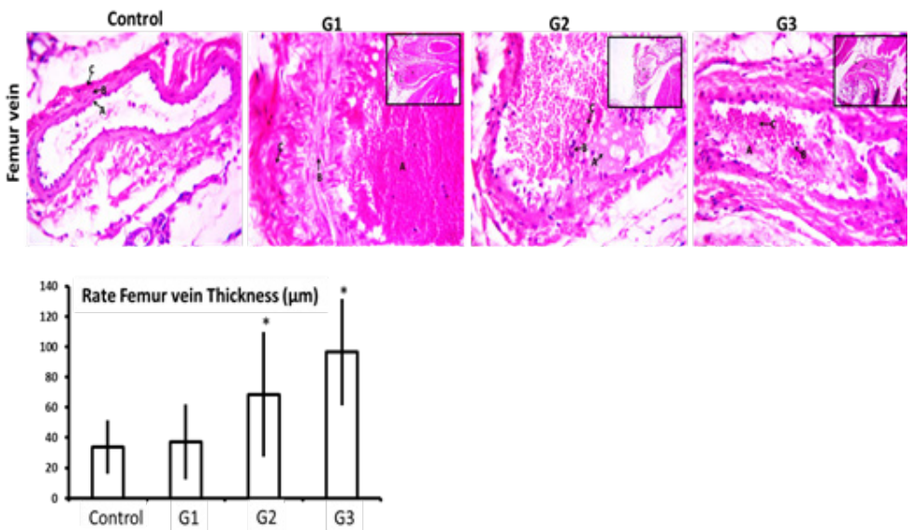

Fig. 2: A representative image for rats' femoral vein tissues in the control group versus dienogest treated groups over three-time points (after 10 days exposure (G1), after 20 days exposure (G2), and after 30 days exposure) with femoral vein thickness increasing after exposure to dienogest. Small box 100µm and large 400µm. Eosin-hematoxylin stain. Small inserted images 100X, large images 400X

DISCUSSION

The present study confirmed that dienogest increases the markers of initiation of clot formation represented by the histological finding of rat femoral vein and vein thickness score confirmed by lung tissue histological finding and inflammation score. The results also showed that the scores of inflammation and vein thickness increased with the duration of exposure to dienogest, represented by an increased score of inflammation and vein thickness in G3 more than in other groups. Many drugs in clinical use have shown adverse drug reactions, hence replaced by alternatives more effective with fewer adverse effects[20].

Acne, hirsutism, and weight gain were the initial challenges associated with COC pills during their initial introduction to the market. Desogestrel, gestodene, and norgestimate's lower androgenic activity have undoubtedly made it possible to lessen these side effects, however, epidemiological studies confirmed that they increase the risk of VTE[21-24]. These studies were confirmed by an alternative meta-analysis study conducted on third-generation COC pills[25]. These outcomes are in the line with the present study.

Additionally, COCs containing EE coupled with drospirenone and cyproterone acetate showed the same effect. The latter two progestins, in contrast to levonorgestrel, desogestrel, gestodene, and norgestimate, have no androgenic or glucocorticoid effects whatsoever[26]. The best contender to treat severe acne and hirsutism in

women is cyproterone acetate because it has the greatest antiandrogenic activity[27]. The chemical composition of drospirenone, which is produced from spironolactone and has an antimineralocorticoid function, sets it apart from other progestogens.

This enables weight reduction prevention throughout COC treatment by offsetting the water retention brought on by estrogens[28]. The creation of these two progestogens with antiandrogenic and antimineralocorticoid properties gave rise to molecules that were closer to progesterone, allowing us to reduce the aforementioned side effects. On the other hand, they were linked with an increased incidence of VTE when combined with EE[29,30].

It was proposed that progestogen molecules may well be involved in the formation of thrombi since findings revealed that COCs with the same estrogen dose, but various progestogens were related to varied VTE incidence. The variation in VTE incidence for each COC, nevertheless, could still be attributable to a unique modulation of the prothrombotic action of EE, exerted by the progestogens, as progestin-only contraceptives do not interfere with the production of clotting proteins[9,31]. In our study, dienogest is a progestin, and hence potentially more thrombogenic than combined estrogen-progestin pills.

The progestogen structure's impact on estrogen receptors, particularly androgen receptors, is really what causes this regulation[32]. Levonorgestrel, which has a potent androgenic effect, partially counteracts the estrogen-dependent modification in hemostasis and hepatic protein synthesis. Levonorgestrel, therefore, counteracts the proangiogenic impact brought on by EE more effectively than desogestrel, gestodene, norgestimate, drospirenone, and cyproterone acetate, which have lesser and even anti-androgenic action[27,33,34].

CONCLUSION

Dienogest, being a progestin only contraceptive, has the potential for increasing the risk of thrombotic markers as indicated by increasing lung inflammation scores in rats with increasing femoral vein thickness indicating oedematous status with venous thrombotic diseases. The effects were boosted by increased inflammatory scores and femoral vein thickness. We do advise the use of combined estrogen-progesterone instead of progestins alone contraceptive.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

خطر التجلط الخثاري الوريدي من استخدام عقار الداينوجست: دراسة تجريبية على الفئران

هديل انور السراج، لقاء خليل الحيالي

كلية الطب، جامعة الموصل، الموصل، العراق

المقدمة والاهداف: هدفت الدراسة إلى تحديد آثار عقار الداينوجست على تحفيز الجلطات الدموية في الفئران بعد التعرض لعقار الداينوجست لمدة ١٠ أيام (دورتي شبق) ، ٢٠ يوما (٤ دورات شبق) ، و ٣٠ يوما (٦ دورات شبق).
طرق العمل: للقيام بذلك ، تم تقسيم ٤٠ فأرا إلى ٤ مجموعات. مجموعة علاج الغفل بدون علاج، وثلاث مجاميع تم تعريضها لعقار الداينوجست (لمدة ١٠ أيام (G١)، و ٢٠ يوما (G٢)، و ٣٠ يوما (G٣)). ثم تم جمع الرئتين والأوردة الفخذية من الجرذان بعد قتلهم، وتم تحضير هذه الأنسجة للتحليل النسيجي.
النتائج: أظهرت النتائج أن الجلطات الدموية الوريدية زادت مع العلاج بعقار الداينوجست كما هو موضح في زيادة درجة الالتهاب في أنسجة الرئة، إلى جانب زيادة سمك جدار الوريد الفخذي، والنتائج النسيجية لترسب الفيبرين، واحتقان الأوعية، وتسلل الخلايا الالتهابية، والتقشر الظهاري.
الاستنتاج: باختصار، يمكن أن يكون العلاج على المدى الطويل خطرا على أمراض التخثراوالامراض القلبية، وبالتالي فإننا ننصح باستخدام البروجسترون البديل أو إضافة جرعة منخفضة من هرمون الاستروجين لتقليل الآثار السامة في المقام الأول عند استخدامه كعلاج بديل بعد انقطاع الطمث.