Published in : Contraception(2020), Vol. 102, Issue 6, pp. 396-402
DOI: 10.1016/j.contraception.2020.08.015
Status : Postprint (Author's version)

EVALUATION OF THE EFFECT OF A NEW ORAL CONTRACEPTIVE CONTAINING ESTETROL AND DROSPIRENONE ON HEMOSTASIS PARAMETERS

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Keywords:
Estetrol
Drospirenone
Ethinylestradiol
Levonorgestrel
Hemostasis
Contraception
Activated protein C resistance

Abstract

Objective: To assess the effect on hemostasis parameters of a new combined oral contraceptive (COC). Study design: In this randomized, single centre, open-label, exploratory study, healthy women received either 15 mg estetrol/3 mg drospirenone (E4/DRSP) (n = 39), 30 mcg ethinylestradiol/150 mcg levonorgestrel (EE/LNG) (n = 30), or 20 mcg ethinylestradiol/3 mg drospirenone (EE/DRSP) (n = 32) for six 28day cycles. Blood was collected at baseline, cycle 3, and cycle 6. Median change from baseline was evaluated for procoagulant, anticoagulant, and fibrinolytic parameters, and for sex hormone-binding globulin (SHBG).

Results: Median change of endogenous thrombin potential (ETP) based activated protein C sensitivity resistance (APCr) at cycle 6 was +30% for E4/DRSP, +165% for EE/LNG (p-value <0.05 vs E4/DRSP), and +219% for EE/DRSP (p-value <0.05 vs E4/DRSP). Changes to prothrombin fragment 1 + 2 and SHBG for E4/DRSP, EE/LNG, and EE/DRSP were +23%, +71%, and +64% (p-value <0.05 vs E4/DRSP); and +55%, +74% and +251% (p-value <0.05 vs E4/DRSP), respectively. At cycle 6, changes to other hemostasis parameters for E4/DRSP were similar or smaller than for EE/LNG or EE/DRSP.

Conclusions: In this study, changes in hemostasis parameters after treatment with 6 cycles of E4/DRSP were smaller or similar to those observed for EE/LNG. Similar, but more pronounced changes were also observed versus EE/DRSP, which supports the hypothesis that the effect of COCs on hemostasis parameters is mainly mediated by the estrogenic component. Further studies are needed to provide more insight into the venous thromboembolic risk of E4/DRSP.

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Implications statement: This study reports that the effects on hemostasis parameters of a COC containing 15 mg E4/3 mg DRSP are less or similar to those for EE/LNG or EE/DRSP. It also demonstrates that the choice of estrogen modulates the effects of COCs on hemostasis parameters.

1. Introduction

Until the mid-1990s, the effects of combined oral contraceptives (COCs) on hemostasis and associated venous thromboembolism (VTE) risk were poorly understood. Discovery of activated protein C resistance (APCr) was an important step forward in understanding the etiology of VTE [1]. Inherited APCr, caused by a mutation in factor V called factor V Leiden (FV Leiden), increases the VTE risk by ~4-fold [2]. In COC users, APCr is also observed, with COCs containing desogestrel (DSG), gestodene (GSD), or drospirenone (DRSP) showing normalized APC sensitivity ratio (nAPCsr) and VTE risk similar to that of heterozygous carriers of FV Leiden [2–5]. Epidemiologic studies have shown that relatively small increases of coagulation factors (e.g. prothrombin and factor VIII), small decreases of anticoagulant proteins (e.g. tissue factor pathway inhibitor [TFPI], protein S and antithrombin) and acquired APCr, can explain the increased VTE risk observed in COC users [6]. The use of COCs containing DSG, GSD, or DRSP in combination with ethinylestradiol (EE) results in more pronounced changes of coagulation markers and a higher observed VTE risk in comparison with EE/levonorgestrel (LNG) [7,8][9]. This difference is likely a result of the weaker antagonism of the EE-induced changes in hemostasis variables with DSG, GSD, and DRSP compared to LNG [10]. Combined oral contraceptives containing the natural adult hormone estradiol (E2) appear to have less effect on hemostasis and recent epidemiological data reported the low VTE risk associated with the combination of E2 valerate with dienogest [9–12].

Estetrol (E4) is a native estrogen produced by the human fetal liver. E4, chemically synthesized from estrone, is identical to the natural hormone and has potential for contraceptive use in humans [13,14]. So far, the hemostatic profile of E4 in combination with 3 mg DRSP has been evaluated with doses up to 10 mg [15]. The current study investigated the hemostatic effects of a 15 mg E4/3 mg DRSP combination, as selected for phase 3 development [16,17].

2. Material and methods

2.1. Study design

This single center, randomized, open-label, controlled, threearm, parallel study in healthy females was conducted from September 2016 through October 2017 at Dinox BV, Groningen, the Netherlands (EudraCT 2016-001316-37, Clinicaltrials.gov NCT02957630). The study, performed in accordance with the Declaration of Helsinki and the ICH E6 (R2) Good Clinical Practice guidelines, was approved by an independent local ethics committee and written informed consent was obtained from all participants before study entry. The study consisted of a pretreatment cycle, followed by six treatment cycles of 28 days. A total of 100 healthy women (40 in the investigational group and 30 per comparator group) was planned to be included in the study. Visits were planned to be at screening, at randomization/baseline, at cycle 3, at cycle 6, and at the end of study.

2.2. Study population

Healthy females aged 18–50 years with a body mass index between 18 and 30 kg/m^2, and a natural menstrual cycle of maximum 35 days were eligible for inclusion. Main exclusion criteria were contraindications for the use

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of hormonal contraceptives, known coagulopathy or thrombogenic mutation, the use of anticoagulants or other drugs affecting coagulation and platelet aggregation, and an abnormal Papanicolaou smear test. The use of an injectable contraceptive was not allowed within 3–10 months prior to screening, depending on the type of injection. Women with combined hormonal contraceptive use prior to the study had a washout period of one menstrual cycle before the pretreatment cycle. The pretreatment cycle started on the first day of the menstrual cycle following screening or following washout if washout was required.

2.3. Study treatment

Eligible subjects were stratified by previous hormonal contraceptive use (2 cycles or >2 cycles without use before study treatment start) and by age (35 years or >35 years of age). Subjects were then assigned, using a computerized random allocation sequence, to one of the following treatments in a 4:3:3 ratio: 15 mg E4 (as monohydrate, equivalent to 14.2 mg anhydrate) combined with 3 mg DRSP (E4/DRSP; 24 day active/4 day placebo regimen), 30 mcg EE combined with 150 mcg LNG (EE/LNG; 21 day active/7 day placebo regimen), or 20 mcg EE combined with 3 mg DRSP (EE/DRSP; 24 day active/4 day placebo regimen). E4/DRSP was manufactured by Haupt Pharma, Münster, Germany and provided by Estetra SPRL, an affiliate's company of Mithra Pharmaceuticals, Liège, Belgium. EE/LNG (Melleva 150/30, Leon Farma) and EE/DRSP (Yaz, Bayer Healthcare) were obtained from a local pharmacy. Study treatment started on the first day of the menstrual cycle following the pretreatment cycle. Treatment compliance was verified by the use of a diary and check of returned packages.

2.4. Study assessments and outcome parameters

2.4.1. Evaluation of hemostasis parameters and sex hormone-binding globulin

The primary endpoint included the evaluation of the following coagulation and fibrinolytic parameters: fibrinogen, prothrombin, factor VII, factor VIII, von Willebrand factor, antithrombin, protein S activity, free protein S, protein C, free TFPI, plasminogen, plasminogen activator inhibitor type-1 (PAI-1), tissue plasminogen activator (tPA), ETP-based APCr (expressed as nAPCsr), $_{D}$-dimer and prothrombin fragment 1 + 2. In addition, sex hormonebinding globulin (SHBG) was also measured. Blood samples for measurement of hemostasis parameters and SHBG were collected at baseline and between days 18 and 21 of cycles 3 and 6. Blood was collected in citrate tubes and serum-separating tubes and processed to plasma or serum. Samples were shipped on dry ice to BARC (Gent, Belgium) and QUALIblood laboratories (Namur, Belgium) for analysis. The analytical methods, including reference ranges are presented in the Supplemental Table 1.

2.5. Statistical analysis

Statistical analyses were performed using SAS software for Windows (SAS Institute Inc, Cary, NC, USA). Statisticians were unblinded to treatments. All randomized subjects who received at least one dose of the study medication and had at least one hemostasis assessment on treatment, without any major protocol deviation impacting the endpoints, were included in the analysis (per protocol dataset). Hemostasis parameters were summarized using descriptive statistics (n, mean, standard deviation [SD], minimum, median, maximum, and coefficient of variation [CV]). No formal sample size calculation was performed, and no formal statistical analysis was planned for this exploratory study. However, additional exploratory nonparametric analysis was performed on the absolute change from baseline. Exploration of the difference between results observed at baseline and

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cycle 3 and baseline and cycle 6 in each treatment was performed using a Wilcoxon signed-rank test. A Kruskal–Wallis test was used to assess the homogeneity of the change from baseline among the three treatments, separately at cycle 3 and at cycle 6. Where results of additional analyses were significant, pairwise comparison of the treatments was done using the Dwass–Steel–Critchlow–Fligner procedure. Due to the exploratory nature of the study, no correction for multiplicity was made. All statistical tests were evaluated with a level of significance of 0.05.

3. Results

3.1. Study population

A total of 143 subjects were screened for eligibility of which 101 were randomized, 98 received study treatment (safety population) and 88 subjects completed the study (per protocol hemostasis population) (Fig. 1). A summary of the demographic data presented in Table 1 shows that there were no apparent group differences at baseline. There were no important protocol deviations, including non-compliance issues.

3.2. Hemostasis parameters

The median values at baseline and at cycles 3 and 6, as well as the median percentage change from baseline of the different treatment arms on (i) procoagulant factors, (ii) anticoagulant and fibrinolytic proteins and (iii) functional coagulation tests, markers of ongoing coagulation, and SHBG are reported in Table 2, Table 3, and Table 4, respectively.

Hemostasis parameters and SHBG were evaluated in 34 subjects in the E4/DRSP group, 27 subjects in the EE/LNG group and 30 subjects in the EE/DRSP group. After 6 treatment cycles, E4/DRSP had less pronounced effects than EE/LNG or EE/DRSP on the percentage change from baseline of the ETP-based APCr (+30% versus +165% and +219%), plasminogen (+12% versus +40% and +36%), tPA (7% versus 33% and 40%) and prothrombin fragment 1 + 2 (+23% versus +71% and +64%). For factor VII, protein C, protein S activity, free-protein S, and SHBG, the changes observed for E4/DRSP were comparable to those for EE/LNG but lower than those for EE/DRSP. No significant differences were seen for fibrinogen, prothrombin, factor VIII, von Willebrand factor, antithrombin, free TFPI, and PAI-1 (Tables 2–4). Results were similar after 3 treatment cycles.

4. Discussion

Combined oral contraceptives, depending on the estrogen and progestin content, affect the levels of several coagulation factors (Fig. 2) [7]. However, hemostasis is a finely balanced physiological process and although the changes induced by COCs often remain within the normal range, synergistic effects may switch the hemostasis profile into an hypercoagulable state. This has been confirmed by several studies demonstrating increased thrombin generation after COC use [18]. Known COC-induced hemostatic changes involve levels of fibrinogen, prothrombin, factors VII, VIII, IX, X, XII, antithrombin, protein C, protein S, and TFPI (Fig. 2) [7].

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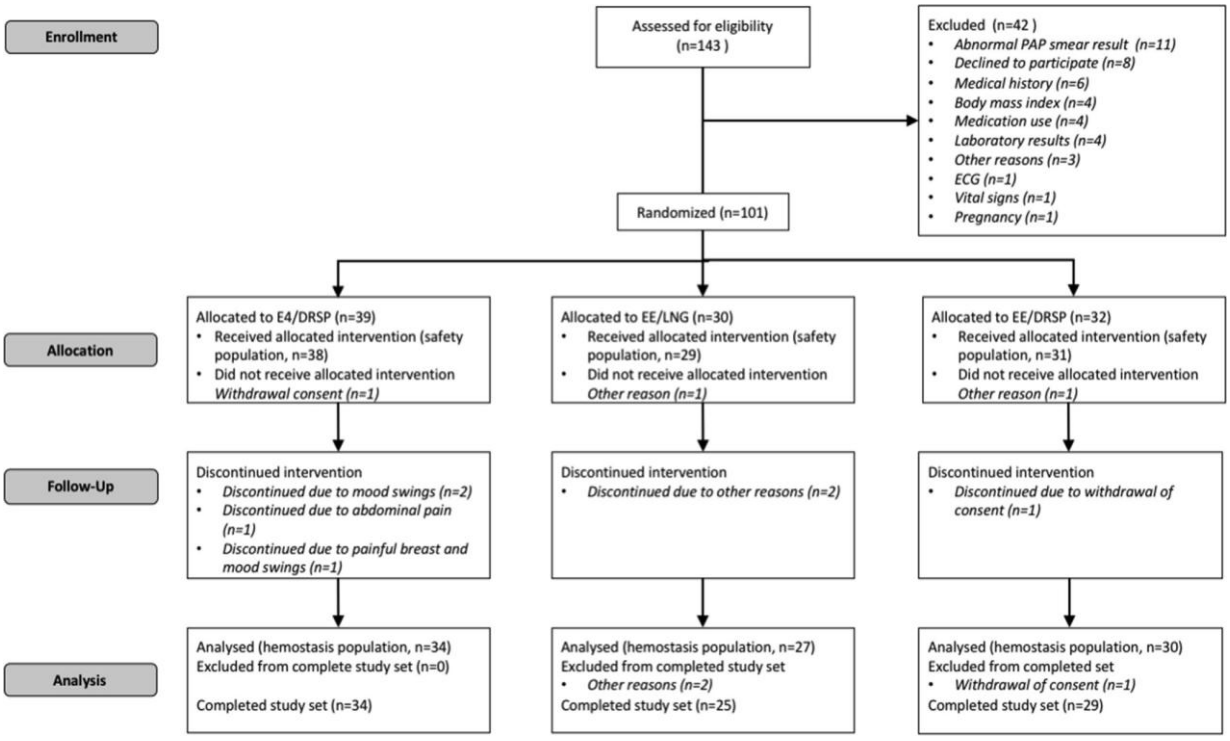

Fig. 1. *Trial flow diagram. DRSP: drospirenone; E4: estetrol; EE: ethinylestradiol; LNG: levonorgestrel; n: number of subjects.*

Table 1. *Mean demographic data at study entry.*

	15 mg E4/3 mg DRSP n = 38	30 mg EE/150 mg LNG n = 29	20 mg EE/3 mg DRSP n = 31	All n = 98
Age, y (range)	26.7 (19–47)	26.2 (18–44)	25.6 (18–40)	26.2 (18–47)
Weight, kg (range)	68.1 (53.1–97.8)	65.6 (50.4–79.2)	63.2 (50.3–80.7)	65.8 (50.3–97.8)
Height, cm (range)	170.8 (159–188)	169.6 (160–181)	168.4 (155–183)	169.7 (155–188)
BMI, kg/m^2 (range)	23.33 (19.2–30.0)	22.83 (18.3–29.8)	22.27 (18.6–26.7)	22.85 (18.3–30.0)

Table 2. *Procoagulant factors: Median (min, max) values at baseline, at cycle 3 and at cycle 6 and changes from baseline (%) at cycle 3 and cycle 6.*

Parameter	Treatment	Baseline	Cycle 3		Cycle 6	
		Value	Value	CFB (%)	Value	CFB (%)
Fibrinogen (mg/dL)	E4/DRSP	240 (173, 355)	258 (153, 372)	3.5 (-23.0, 77.0)	262 (197, 370)	10.0 (-24.0, 54.0)*
	EE/LNG	239 (144, 472)	274 (182, 424)	8.0 (-38.0, 110.0)*,#	280 (168, 430)	5.0 (-35.0, 95.0)
	EE/DRSP	229 (176, 409)	291 (190, 388)	22.0 (-36.0, 69.0)*	280 (225, 436)	16.0 (--44.0, 58.0)*
Prothrombin (%)	E4/DRSP	86 (67, 106)	92 (69, 109)	9.0 (-11.0, 24.0)*	91 (74, 107)	7.0 (-12.0, 26.0)*
	EE/LNG	87 (72, 112)	101 (83, 119)	13.0 (-1.0, 35.0)*,#	96 (84, 129)	13.0 (-7.0, 32.0)*
	EE/DRSP	89 (77, 112)	101 (88, 125)	14.5 (1.0, 33.0)*,#	101 (81, 117)	7.0 (-4.0, 49.0)*

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Factor VII (%)	E4/DRSP	94 (61, 166)	92 (54, 133)	-4.0 (-30.0, 26.0)*	95 (68, 141)	-3.0 (-31.0, 44.0)
	EE/LNG	93 (53, 127)	83 (56, 118)	-7.0 (-41.0, 43.0)*,#	80 (53, 128)	-5.0 (-38.0, 47.0)*,#
	EE/DRSP	94 (64, 130)	116 (56, 187)	18.5 (-16.0, 65.0)*	113 (64, 178)	20.0 (-14.0, 49.0)*
Factor VIII (%)	E4/DRSP	114 (85, 255)	135 (90, 268)	11.0 (-28.0, 58.0)*	125 (80, 226)	5.0 (-33.0, 74.0)
	EE/LNG	115 (74, 217)	126 (90, 231)	9.0 (-35.0, 62.0)	112 (70, 209)	3.0 (-38.0, 79.0)
	EE/DRSP	110 (72, 203)	136 (80, 226)	20.5 (-15.0, 56.0)*	118 (68, 217)	9.0 (-33.0, 66.0)
Von Willebrand factor (%)	E4/DRSP	103 (60, 240)	106 (70, 212)	5.0 (-21.0, 39.0)*	108 (63, 202)	5.0 (-26.0, 52.0)
	EE/LNG	96 (56, 295)	94 (52, 266)	-2.0 (-41.0, 33.0)	97 (44, 263)	-2.0 (-41.0, 38.0)
	EE/DRSP	88 (55, 154)	94 (60, 167)	7.5 (-15.0, 42.0)*	96 (62, 192)	13.0 (-19, 51.0)*

CFB = Change from baseline. 1 Data at Cycle 6 or end of treatment. *Different versus baseline, $p < 0.05$ using a signed rank test. # Different from treatment with 15 mg E4/3 mg DRSP, $p < 0.05$ using the Dwass–Steel–Critchlow–Fligner test.

Table 3. *Anticoagulant proteins and fibrinolytic proteins: Median (min, max) values at baseline, cycle 3 and cycle 6 and changes from baseline (%) at cycle 3 and cycle 6.*

Parameter	Treatment	Baseline	Cycle 3		Cycle 6[1]	
		Value	Value	CFB (%)	Value	CFB (%)
Anticoagulant proteins						
Antithrombin (%)	E4/DRSP	96(87, 110)	97 (87, 113)	0.0 (11.0, 13.0)	98 (85, 113)	-1.0 (9.0, 13.0)
	EE/LNG	99 (64, 124)	96 (64, 112)	-2.0 (17.0, 14.0)*	94 (66, 117)	-5.0 (14.0, 13.0)*
	EE/DRSP	97(88, 114)	96 (83, 107)	-2.5 (13.0, 14.0)	97 (83, 116)	-3.5 (17.0, 16.0)
Protein S activity (%)	E4/DRSP	93 (74, 134)	92 (72, 145)	1.0 (22.0, 33.0)	89 (69, 126)	-4.0 (23.0, 30.0)
	EE/LNG	105 (82, 142)	101 (80, 145)	-2.0 (32.0, 59.0)	96 (79, 133)	-5.0 (29.0, 35.0)*
	EE/DRSP	104 (65, 149)	80 (55, 109)	-26.0 (41.0, 6.0)*,#	74 (51, 106)	-30.5 (45.0, 8.0)*,#
Protein S free (%)	E4/DRSP	84 (59, 111)	90 (70, 114)	8.0 (10.0, 34.0)*	87 (61, 117)	5.0 (19.0, 32.0)
	EE/LNG	94 (76, 121)	99 (70, 136)	2.0 (27.0, 66.0)	92 (71, 125)	-3.0 (24.0, 29.0)*
	EE/DRSP	88 (69, 134)	71 (53, 103)	-21.0 (36.0, 0.0)*,#	69 (49, 88)	-22.5 (46.0, 1.0)*,#
Protein C (%)	E4/DRSP	95 (73, 141)	94 (73, 137)	1.0 (14.0, 32.0)	91 (72, 140)	2.0 (19.0, 26.0)
	EE/LNG	98 (71, 135)	106 (83, 142)	12.0 (11.0, 34.0)*,#	101 (75, 137)	7.0 (14.0, 39.0)*
	EE/DRSP	98 (69, 122)	111 (88, 171)	19.5 (9.0, 46.0)*,#	114 (87, 183)	17.5 (0.0, 56.0)*,#
TFPI free (U/mL)	E4/DRSP	1.1 (0.5, 1.6)	1.0 (0.6, 1.6)	9.0 (56.0, 74.0)	1.0 (0.6, 1.6)	-8.5 (53.0, 52.0)
	EE/LNG	1.0 (0.7, 1.6)	1.1 (0.6, 1.6)	-3.0 (46.0, 100.0)	1.0 (0.6, 1.6)	-6.0 (58.0, 63.0)
	EE/DRSP	1.0 (0.7, 1.6)	1.0 (0.6, 1.6)	-19.0 (58.0, 98.0)	1.0 (0.5, 1.3)	-20.0 (49.0, 30.0)*
Fibrinolytic proteins						
Plasminogen (%)	E4/DRSP	94 (68, 123)	107 (72, 126)	12.0 (12.0, 31.0)*	108 (82, 131)	12.0 (5.0, 35.0)*
	EE/LNG	96 (78, 127)	135 (111,162)	45.0 (13.0, 65.0)*,#	136 (110, 173)	40.0 (15.0, 80.0)*,#
	EE/DRSP	99 (78, 123)	131 (111,162)	32.0 (9.0, 78.0)*,#	132 (114, 172)	35.5 (6.0, 68.0)*,#
PAI-1 (U/mL)	E4/DRSP	0.9 (0.5, 5.5)	1.1 (0.5, 3.3)	0.0 (68.0, 560.0)	1.3 (0.5, 3.7)	20.0 (68.0, 400.0)
	EE/LNG	0.5 (0.5, 6.2)	0.5 (0.5, 2.5)	0.0 (92.0, 400.0)	0.5 (0.5, 1.5)	0.0 (92.0, 200.0)
	EE/DRSP	0.7 (0.5, 6.2)	0.5 (0.5, 6.2)	0.0 (89.0, 120.0)*	0.6 (0.5, 5.2)	0.0 (87.0, 240.0)
t-PA (ng/mL)	E4/DRSP	4.5 (2.0, 10.6)	3.8 (2.2, 6.3)	16.0 (51.0, 104.0)*	4.1 (2.1, 8.3)	7.0 (52.0, 79.0) ,#
	EE/LNG	4.3 (1.9, 12.7)	2.7 (1.6, 4.7)	35.0 (74.0, 34.0)*	2.8 (1.2, 4.4)	33.0 (84.0, 29.0)*,#
	EE/DRSP	4.4 (1.5, 13.1)	2.9 (1.8, 8.7)	35.5 (70.0, 93.0)*	2.8 (1.0, 7.0)	39.5 (72.0, 80.0)*

TFPI = tissue factor pathway inhibitor; PAI-1 = plasminogen activator inhibitor-1; t-PA = tissue plasminogen activator; CFB = Change from baseline. [1]Data at Cycle 6 or end of treatment. *Different versus baseline, $p < 0.05$ using a signed rank test. # Different from treatment with 15 mg E4/3 mg DRSP, $p < 0.05$ using the Dwass–Steel–Critchlow–Fligner test.

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4.1. Impact of E4/DRSP on hemostasis biomarkers

Changes in levels of procoagulant proteins were small for E4/ DRSP and EE/LNG. For some subjects in the EE/DRSP group, levels of FVII slightly exceeded the threshold that is used to classify subjects with a higher thrombotic risk (i.e. >170% of FVII levels observed in the normal population), although there is little evidence suggesting that a greater FVII level is a risk factor for VTE [19]. None of these subjects had FVII levels above the threshold at baseline (Table 2).

No relevant changes were observed for the anticoagulant factors antithrombin, protein S (free and activity), protein C, and TFPI levels with E4/DRSP (Table 3). Levels and activity of protein S were decreased for EE/LNG and EE/DRSP, and in some subjects treated with EE/DRSP this change could almost be qualified as a protein S deficiency (i.e. levels around 50%). However, protein S activity assays have shown decreased protein S levels in 10–15 percent of healthy subjects, and that values may return to normal when the test is repeated on a new sample [20]. The statistically significant decrease of TFPI levels observed for EE/DRSP, i.e. 20% versus 8.5%, and 6% for E4/DRSP and EE/LNG (Table 3), was in line with TFPI levels observed in the literature [3]. Low plasma TFPI is probably a weak risk factor for VTE [22], but it remains an open question whether a reduction of TFPI during COC use is associated with an increased risk of VTE.

Concerning the fibrinolytic system, the change in tPA levels with E4/DRSP was smaller compared with EE/LNG or EE/DRSP (7%, 33%, and 39.5%, respectively). The changes in PAI-1 levels were not different between the 3 treatments (+20%, +0%, and +0%, for E4/DRSP, EE/LNG and EE/DRSP, respectively) while all treatments induced a significant increase in plasminogen levels (+12%, +40%, and +35.5% for E4/DRSP, EE/LNG and EE/DRSP, respectively) (Table 3). These results do not allow any conclusions to be drawn regarding a potential hypo- or hyper-fibrinolytic profile of E4/DRSP but do underline the weak impact of E4/DRSP on fibrinolysis markers. In addition, there is little evidence that changes to fibrinolysis parameters are important risk factors for VTE.

The change in nAPCsr, as determined by the ETP-based APCr, was statistically significant, being lower for E4/DRSP (+30%) in comparison with EE/LNG (+165%), and EE/DRSP (+219%) (Table 4). Activated protein C resistance, determined with an ETP-based assay, has been considered to be a suitable marker to assess the thrombogenicity of COCs for two decades [12,20] and the extent of ETP-based APCr is related to the thrombotic risk of newer COC preparations, the contraceptive patch, hormone replacement therapy, pregnancy, and other VTE risk states [23,24]. In addition, a relationship between an increase in the nAPCsr and the risk of VTE was reported by Morimont et al. [25]. In their study, mean nAPCsr values for different COC combinations ranged from 3.75 to 5.40 while in non-users the mean nAPCsr (±SD) was 1.68 (±0.88) [25]. Modeling showed that these nAPCsr values were exponentially related to the relative VTE risk of COCs seen in epidemiological studies (r-square = 0.95), but clearly prospective data are needed to confirm the predictive value of the nAPCsr for the assessment of VTE risk.

The increase of D-dimer was relatively small (+4%, +7% and +0% for E4/DRSP, EE/LNG and EE/DRSP, respectively). The tight dispersion around the median value at baseline in the E4/DRSP group compared with the high dispersion in the EE/LNG and EE/DRSP groups may explain the significance observed for E4/DRSP, which was not observed for EE/LNG, despite the numerically higher values for the latter. D-dimer is mainly used for diagnosing patients who present with a low-medium clinical probability of having VTE, and many variables may increase the D-dimers transiently. Therefore, changes in D-dimer should not be regarded as predictive of any thrombotic risk [21]. Prothrombin fragment 1 + 2, which represents a direct marker of ongoing coagulation since it results in the catalytic conversion of inactive prothrombin into active thrombin, was increased for E4/DRSP (+23%) but this increase was significantly lower in comparison with EE/LNG (+71%) and EE/DRSP (+64%). However, the absolute changes were low and show high dispersion, especially in the EE/LNG arm (Table 4).

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Table 4. *Functional coagulation tests, markers of ongoing coagulation and SHBG: Median (min, max) values at baseline, cycle 3 and cycle 6 and changes from baseline (%) at cycle 3 and cycle 6.*

Parameter	Treatment	Basdeline	Cycle 3		Cycle 6[1]	
		Value	Value	CFB (%)	Value	CFB (%)
Functional coagulation tests						
APC resistance, ETP-based (nAPCsr)	E4/DRSP EE/LNG EE/DRSP	1.67 (0.53, 3.35) 1.49 (0.00, 5.03) 1.37 (0.46, 3.06)	2.31 (0.81, 4.12) 3.61 (1.74, 6.67) 4.36 (2.64, 6.13)	39.5 (19, 117.0)* 165.0 (33.0, 496.0)*,# 229.0 (91.0, 781.0)*,#	2.09 (0.80, 4.28) 3.35 (0.73, 7.39) 4.50 (2.54, 5.85)	30.0 (53.0, 233.0)* 164.5 (30.0, 424.0)*,# 218.5 (99.0, 763.0)*,#
Markers for ongoing coagulations						
D-dimer (ug/mL FEU)	E4/DRSP EE/LNG EE/DRSP	0.27 (0.27, 0.43) 0.27 (0.27, 2.28) 0.27 (0.27, 1.14)	0.27 (0.27, 0.86) 0.31 (0.27, 1.82) 0.28 (0.27, 1.43)	0.0 (36.0, 219.0) 0.0 (65.0, 59.0) 0.0 (46.0, 93.0)	0.29 (0.27, 0.59) 0.33 (0.27, 1.19) 0.31 (0.27, 0.93)	4.0 (33.0, 97.0)* 7.0 (84.0, 93.0) 0.0 (33.0, 93.0)
Prothrombin fragment 1 + 2 (nmol/L)	E4/DRSP EE/LNG EE/DRSP	0.133 (0.060, 0.261) 0.111 (0.060, 0.332) 0.114 (0.073, 0.0260)	0.129 (0.064, 0.284) 0.169 (0.098, 0.343) 0.169 (0.095, 0.416)	7.0 (39.0, 73.0) 62.0 (2.0, 125.0)*,# 47.5 (6.0, 187.0)*,#	0.151 (0.086, 0.360) 0.184 (0.118, 0.818) 0.191 (0.094, 0.377)	23.0 (34.0, 108.0)* 71.0 (27.0, 357.0)*,# 64.0 (1.0, 127.0)*,#
SHBG						
SHBG (nmol/L)	E4/DRSP EE/LNG EE/DRSP	64.8 (25.3, 117.9) 67.3 (27.1, 144.4) 70.6 (36.2, 125.6)	97.0 (45.5, 185.7) 118.6 (58.5, 187.6) 245.9 (164.0, 382.0)	51.5 (23.0, 132.0)* 67.0 (10.0, 313.0)* 239.5 (128.0, 608.0)*,#	87.2 (52.7, 196.0) 119.8 (65.2, 191.4) 264.3 (162.3, 447.4)	55.0 (22.0, 171.0)* 74.0 (17.0, 261.0)* 251.0 (122.0, 637.0)*,#

APC = activated protein C; ETP = endogenous thrombin potential; SHBG = sex hormone-binding globulin; CFB = Change from baseline; TF, tissue factor; TFPI, tissue factor pathway inhibitor; t-PA, tissue plasminogen activator inhibitor; vWF, von Willebrand Factor. [1]Data at Cycle 6 or end of treatment. *Different versus baseline, $p < 0.05$ using a signed rank test. # Different from treatment with 15 mg E4/3 mg DRSP, $p < 0.05$ using the Dwass–Steel–Critchlow–Fligner test.

4.2. Differential effect of estetrol and ethinylestradiol on hemostasis and liver biomarkers

EE/DRSP has been associated with a higher risk of VTE than EE/ LNG [5]. The use of E4 instead of EE in combination with DRSP appears to reduce the effect on hemostatic parameters, including the nAPCsr [15]. E4/DRSP was reported to have a neutral profile on the liver compared to EE/DRSP [22]. This also translated into a minimal effect on triglycerides, angiotensinogen and SHBG, although tested at lower E4 doses than in this study [23]. The mild increase of SHBG observed with E4/DRSP, which was significantly less than the change of SHBG with EE/DRSP, suggests a lower estrogenic effect of E4 on the liver. The lower effect observed with EE/ LNG compared with EE/DRSP is explained by the androgenic properties of LNG, which are absent with DRSP [10]. This enables LNG to counteract the effects of EE on the liver, thereby reducing total estrogenicity and thrombogenicity [4,7].

Published in : Contraception(2020), Vol. 102, Issue 6, pp. 396-402
DOI: 10.1016/j.contraception.2020.08.015
Status : Postprint (Author's version)

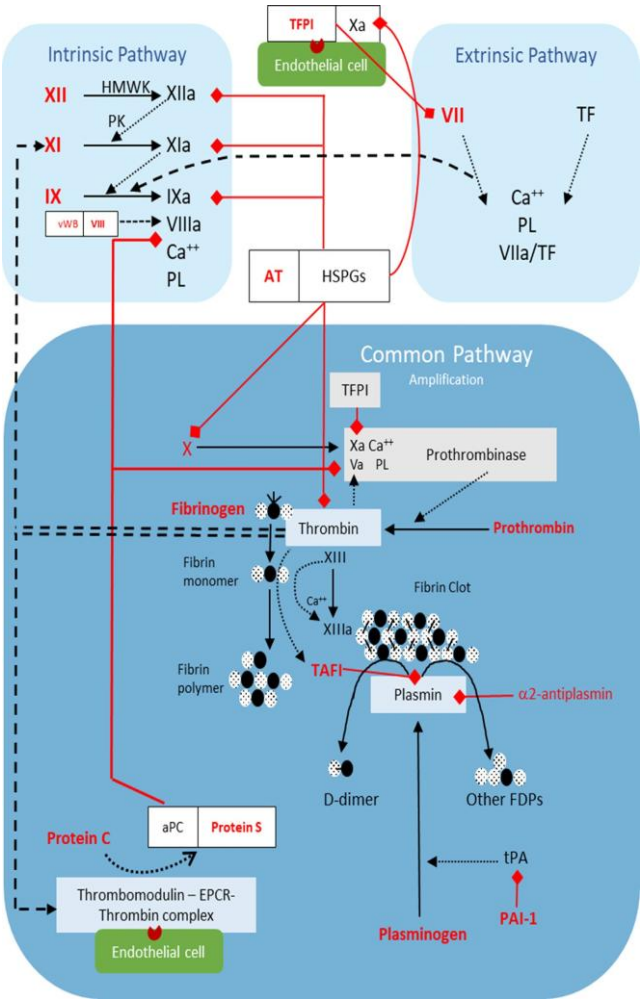

Fig. 2. *A simplified scheme of the coagulation and fibrinolysis pathways. Factor or proteins in orange are those suspected to be altered by COC treatments. Dashed black lines represent activation while black lines represent factor or protein activation. Red lines represent inhibitory actions. Abbreviations: aPC, activated protein C; AT, antithrombin; Ca^{++}, calcium; EPCR, endothelial protein C receptor; FDP, fibrin degradation product; HMWK, high molecular weight kininogen; HSPG, heparan sulfate proteoglycan; PAI-1, plasminogen activator inhibitor-1; PK, pre-kallikrein; PL, phospholipids; TAFI, thrombin activatable fibrinolysis inhibitor*.

4.3. Strengths and limitations

This study was conducted with a limited number of subjects with no former sample size calculation. A sample size of 30–40 patients per treatment arm was chosen since several studies have shown that this study size was sufficient to estimate clinically meaningful changes of hemostasis parameters during COC use. Due to the exploratory nature of the study, no correction for multiplicity was made. A classical Bonferroni adjustment would have resulted in a significance level of 0.003 (0.05/17), if such adjustment had been performed with the 17 different parameters (coagulation factors and SHBG) assessed in this study. It is worth emphasizing that all values of the pairwise comparisons indicated as $p < 0.05$ were $p < 0.003$ (except for tPA, $p = 0.0046$ for the difference between E4/DRSP and EE/LNG) confirming the robustness of the findings.

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Status : Postprint (Author's version)

5. Conclusion

In this study, changes in hemostasis parameters after treatment with 6 cycles of E4 DRSP were smaller or similar to those observed for EE/LNG. More pronounced changes were observed versus EE/ DRSP, which supports the hypothesis that the effect of COCs on hemostasis parameters is mainly mediated by the estrogenic component. The neutral profile of E4/DRSP on hemostasis parameters suggests that it is less likely to be associated with VTE risk, but this has to be confirmed by data obtained from post-marketing surveillance program assessing the clinical occurrence of VTE events.

Acknowledgements

The study was sponsored by Estetra SPRL, an affiliate's company of Mithra Pharmaceuticals, Liège, Belgium. Medical writing support was provided by Mireille Gerrits PharmD, at Terminal 4 Communications, Hilversum, the Netherlands. Statistical support was provided by Pharmalex Belgium, Mont-Saint-Guibert, Belgium.

Conflict of interest

J. Douxfils is the director and founder of Qualiblood, a contract research organization that received funding from Mithra for the conduct of the study. He also reports personal fees from Daiichi-Sankyo, Diagnostica Stago, Portola, Roche and Roche Diagnostics. J. Rosing has provided expert witness testimony relating to effects of hormonal contraceptives on blood coagulation and his laboratory executed several industry-sponsored studies on the effects of female sex hormones on coagulation. He reports personal fees from Mithra and from Pantharei Bioscience and Oncology.C. Klipping and I. Duijkers are directors of Dinox BV, a contract research organization that received funding from Mithra for the conduct of the study. V. Kinet, M. Mawet, C. Maillard and M. Jost are employees of Mithra. JM Foidart is a member of the board at at Mithra and received financial support for the supervision of this study.

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Published in : Contraception(2020), Vol. 102, Issue 6, pp. 396-402
DOI: 10.1016/j.contraception.2020.08.015
Status : Postprint (Author's version)

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