Supplementary Online Content

Salem J-E, Dureau P, Bachelot A, et al. Association of oral contraceptives with drug-induced QT interval prolongation in healthy nonmenopausal women. *JAMA Cardiol*. Published online August 1, 2018. doi:10.1001/jamacardio.2018.2251

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. *Methods.*

(References at the end of this document)

Study design

The GENEREPOL study[1] (clinical trials.gov: NCT00773201) was an open-label prospective study where healthy volunteers were challenged with 80 mg sotalol oral intake in a Genome Wide Association Study of genetic factors associated with IKr inhibition evaluated on the electrocardiogram (ECG). From February 2008 to February 2012, 995 healthy volunteers were enrolled in this study. Duration of participation in this study was one day. Inclusion criteria were male or female, aged between 18 and 60 years, only of European or North African origin, with a body mass index between 19 and 29 kg/m² and able to give an informed consent. Exclusion Criteria were pregnancy, asthma, resting heart rate below 50 bpm, abnormal ECG (including right bundle branch block) or QRS>100msec, systolic blood pressure<100 mmHg, history of atrioventricular block or Raynaud phenomenon, known chronic illness such as cardiac or renal insufficiency with chronic treatment, QT prolonging drug or any chronic treatment except for contraceptive pills, family or personal history of congenital long QT syndrome, arrhythmia or sudden death and QTc Fridericia (QTcF) >450ms. The study protocol and all methods applied were approved by the Committee for the Protection of Human Subjects of Paris Ile de France V (Paris, France) and prior written informed consent was obtained from all subjects after being fully informed regarding the nature and risks of the study. The study complied with the principles of the international conference on harmonization guidelines on good clinical practice and of the world medical association declaration of Helsinki.

Volunteers were hospitalized at 8:00am for a duration of approximatively 6 hours at the Clinical Investigation Centre Paris-Est (Saint-Antoine and Pitié-Salpêtrière Hospital, Paris, France) after an overnight fast. An intravenous catheter was inserted for blood collections; then, a continuous digital 12-lead ECG recording monitoring was started using a Cardioplug device (Cardionics Inc®, Brussels, Belgium) connected to a personal computer. Baseline ECG

recordings (triplicate of 10-seconds each) were obtained after the subjects had rested for at least 10 minutes in the supine position. Each subject was then given a single oral dose of sotalol (80mg) and ECG monitoring was continuously pursued. Three hours post dosing (H3), 10-second ECG recordings were again extracted (triplicate) after the subjects had rested for at least 10 minutes in the supine position before lunch. At H3, a blood sample was drawn for the determination of plasma sotalol concentration. The participants were finally discharged 5 to 6 hours post dosing after verifying that their QTcF was < QTcF baseline + 40 msec.

Among all subjects included, 615 were women. Type, dose and timing of their chronic contraceptives modalities was left at the discretion of the treating physician and were unchanged for the purpose of this study.

The present work focuses on the impact of different oral progestin-derived contraceptive pills on sotalol-induced QTc prolongation and electrocardiographic changes suggestive of IKr-inhibition as a function of their androgenic potency. Therefore, only non-menopausal women taking no hormonal contraception, or receiving chronically any of the following oral derived contraceptive pills (OC) were considered for analysis: levonorgestrel (2^{nd} generation, high androgenic potency), desogestrel or gestodene (3^{rd} generation, intermediate androgenic potency) and drospirenone (partial antiandrogenic action, classified as 4^{th} or other generation). Subgroups of women taking non-oral hormonal contraception or any other hormone-derived drug (for contraceptive indication or not) were too small (n/subgroup: 2.5 [1.75-7.25]; median and interquartile range) and therefore not included in the analysis. The main objective of the study was to compare association of levonorgestrel versus drospirenone with sotalol-induced QT-prolongation because these drugs had the most different androgenic activity (levonorgestrel being androgenic and drospirenone, anti-androgenic) among commonly used and approved oral contraceptive pills.

Electrocardiographic phenotyping

Since drug-induced Long QT syndrome with sotalol represents the equivalent of an induced form of congenital Long QT2 syndrome,[2,3] we evaluated its classical ECG features at baseline and three hours (H3) after sotalol intake. QTc, and the presence of notches were carefully quantified. Fridericia's correction ($QT/RR^{0.33}$) was chosen for QT correction on heart rate.[4,5] QTc provided an accurate correction of QT for heart rate ($r \leq 0.1$ for QTc *vs.* heart rate regression). Analysis of ECGs was performed after inclusion of all subjects using CARDIABASE post-treatment software (Banook Group®, Nancy, France).[1] QTc was measured by the tangent method in Lead II on three consecutive beats and the mean value of a triplicate evaluation was retained.[6,7] For QTc measurements, inter- and intra-observer agreements were periodically assessed using ICC (intra-class correlation coefficient) which was continuously measured >0.9, indicating an excellent agreement (hence interchangeability) and repeatability between operators.

For these quantitative parameters, the change (Δ) between baseline and H3 value was calculated as follow:

$$\Delta \text{ QTc (ms)} = H3\ Mean\ QTc - Baseline\ Mean\ QTc \quad (1)$$

$$\Delta \text{ QTc (\%)} = \left(\frac{H3\ Mean\ QTc - Baseline\ Mean\ QTc}{Baseline\ Mean\ QTc}\right) * 100 \quad (2)$$

The assessment of notching was performed independently by two experienced observers (JES and CFB). A subject was considered as "notcher" when both observers agreed on the presence of a notch.[1] Concordance between observers was excellent ($\kappa = 0.92 \pm 0.04$).

All electrocardiographic analyses were performed blinded to any of the women's characteristics, including modalities of contraceptive treatment.

eTable 1. *Characteristics of the different hormonal oral contraceptive pills.*

	Levonorgestrel	Desogestrel	Gestodene	Drospirenone
Number of subjects	137	41	51	62
Concomitant EE use* (%)	99	78	100	100
EE min/max dosing** (µg/day)	20-35	20-30	15-35	20-30
PG min/max dosing** (µg/day)	30-175	75-150	60-75	3000
PG generation	2nd	3rd		other (4th)
Progestational activity	High	High		Intermediate
Androgenicity	High	Intermediate / Variable		Anti-androgenic

Statistics: * *p<0.05.*

Abbreviations: EE: Ethynil-estradiol, PG: Progestin, **: in case of concomitant use of EE except for days on placebo or no pills.

eTable 2. *Data from the EudraVigilance database (through 05/09/2018) concerning number and type (MedDRA classification) of declared individual case safety reports in women receiving drospirenone and levonorgestrel.*

	Total number of ICSRs	Ventricular arrhythmia/ tachycardia	Ventricular Fibrillation	Torsade de Pointes	Ventricular arrhythmia (sum)	Cardiac (respiratory) arrest	Apparent Death	Sudden (cardiac) death	Cardiac death (Sum)	Long QT Syndrome/ ECG QT prolonged
Drospirenone - Ethynil-Estradiol	32848	13	10	1	24	135	5	6	146	6
Drospirenone - Estradiol	595	0	0	0	0	0	0	0	0	0
Total Drospirenone	**34784**	13	10	1	**24**	135	5	6	**146**	**6**
Levonorgestrel	64459	4	4	0	8	13	2	4	19	2
(Estriol), Estradiol - Levonorgestrel	408	0	0	0	0	0	0	0	0	0
Ethynil Estradiol - Levonorgestrel	7206	2	4	1	7	25	0	1	26	0
Total Levonorgestrel	**72073**	6	8	1	**15**	38	2	5	**45**	**2**

ECG: Electrocardiogram
ICSRs: individual case safety reports
Unspecified Gender was considered women
Column and line in bold were considered for statistical analysis

eReferences. Bibliography

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