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Case Report

Drospirenone detected in postmortem blood of a young woman with pulmonary thromboembolism: A case report and review of the literature

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ABSTRACT

Progestin/estrogen oral contraceptives have some side effects, including venous thromboembolism. To alleviate side effects, improvements have been made to low-dose oral contraceptives, including reductions in the amount of estrogen and/or changes the type of progestin. A compound drug containing 3 mg drospirenone and 20 μg ethinylestradiol (DRSP/EE20, YAZ®) was released in overseas markets in 2006, and in Japan in 2010 as a newly developed low-dose medicines. This drug is expected to have lower side effects.

We received a medicolegal autopsy case of a young woman who had been prescribed YAZ for dysmenorrhea for 17 months. The autopsy revealed a blood clot in her pulmonary artery bifurcation. Blood screening by ultra-performance liquid chromatography–mass spectrometry analysis did not detect any medicinal toxicants. However, from police investigations, it is strongly believed that she had been taking YAZ. Therefore we performed a single ion resolution mode assay and detected DRSP. A quantitative analysis revealed 32.3 ng/mL of DRSP. As no other cause of the pulmonary thromboembolism was evident, we consider YAZ as the likely cause of the pulmonary thromboembolism.

Recent reports from the past few years suggest a higher risk of venous thromboembolism with DRSP/EE20 than earlier progestin/estrogen oral contraceptives. Comparing the risk associated with DRSP/EE20 and DRSP/EE30, one report found no differences and another report showed DRSP/EE20 was associated with a higher risk than DRSP/EE30. No cases of thrombosis caused by progestin alone have been reported. But comparing the risk between DRSP/EE20 and other progestins/EE20, two studies reported DRSP/EE20 had a higher risk than other progestins/EE20. The incidence of venous thromboembolism is highest in the first year of use and decreases thereafter. Because DRSP/EE20 has been on the market for only a couple of years, it is necessary for clinicians to use the drug carefully and accumulate more side-effect data. It is important for forensic scientists to confirm all of the prescribed drugs in autopsy cases, search the risks of identified drugs, particularly new drugs, and provide relevant case information in a timely manner.

1. Introduction

Side effects of progestin/estrogen oral contraceptives include headache, nausea, atypical genital bleeding, hirsutism, acne, weight gain, and rarely venous thromboembolism [1–8]. To alleviate these side effects, improvements have been made to low-dose oral contraceptives, including reductions in the amount of estrogen and/or changes the type of progestin.

As a newly developed low-dose pill, a compound drug containing 3 mg drospirenone and 30 μg ethinylestradiol (DRSP/EE30 in 21 pills plus 7 placebo pills; Yasmin®; Bayer HealthCare Pharmaceuticals, Inc., Berlin, Germany) was released to overseas markets in 2000. This drug is expected to have lower side effects (e.g., acne, hirsutism, weight gain, and blood pressure elevation) because of the anti-androgen action and anti-mineralocorticoid activity of DRSP (3-Oxo-6β,7β:15β,16β-dimethano-17α-pregn-4-ene-21,17-carbolactone; $C_{24}H_{30}O_3$; 366.49 kDa), which are absent in conventional progestational hormones. As a measure against thrombosis, one of the side effects of estrogen, the amount of EE was later reduced from 30 μg to 20 μg, and at the same time drug holidays were reduced from 7 days to 4 days to prevent changes in hormonal levels on these days. This new version of the drug containing 3 mg DRSP and

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20 μg EE (DRSP/EE20 in 24 pills plus 4 placebo pills; YAZ®; Bayer HealthCare Pharmaceuticals, Inc.) has been available since 2006 on the overseas market and since 2010 in Japan, where the pill was approved for the treatment of dysmenorrhea, but not as an oral contraceptive. In addition to its efficacy for contraception, dysmenorrhea, premenstrual symptoms, and premenstrual mood disorder [9–13], DRSP/EE20 has few side effects such as acne and weight gain [11,12,14–16]. A string of recent large-scale studies, however, have shown that not only DRSP/EE30 [17–23], but also DRSP/EE20, has a high risk of venous thrombosis compared with conventional low-dose pills [20,24].

We recently encountered a fatal case of a woman in her late teens who had been prescribed YAZ and died of acute pulmonary thromboembolism. DRSP was detected in blood collected at autopsy. Because no other potential causes of death were evident, YAZ was considered to be the primary cause of the pulmonary thromboembolism, thereby making this case the first local case of death due to YAZ. The girl had been prescribed DRSP/EE20 for dysmenorrhea since her initial hospital visit; however, because of the relatively high risk of thrombosis, DRSP/EE20 may have been excluded from the initial drugs of choice when prescribing low-dose pills. In this article, we report the present case and review recent literature on DRSP/EE20.

2. Material and methods

2.1. Case history

A female teenage college student living alone was found dead in her apartment. She was a member of a tennis club and had never smoked or shown abnormalities on medical checkups. Her family history was unremarkable. She had been prescribed YAZ for dysmenorrhea in the 17 months before she died. She had never complained of pain or edema in her legs. Without warning, after a day of visiting an indoor amusement center, doing karaoke, playing sports with her friends for 8 h, and eating dinner before returning home, her responsiveness suddenly declined while chatting with her friends on the Internet. She did not complain of any disorder. Three days later, a superintendent visited her apartment and found her dead, lying on a legless chair. Postmortem lividity and rigor mortis were apparent. Her body marks and clothes suggested that she had died on the day she visited the amusement center. Thirty-six YAZ tablets were found in her room. Medico-legal autopsy was performed 4.5 days after death because the cause of death was equivocal.

2.2. Ultra-performance liquid chromatography–mass spectrometry analysis

A screening assay, a single ion resolution (SIR) mode assay and a quantitative analyses of her postmortem blood were performed by ultra-performance liquid chromatography–mass spectrometry (UPLC–MS). When we performed a SIR mode assay and a quantitative analyses, we targeted DRSP because the Cmax level of DRSP is 1000 times higher than that of EE in the serum.

2.2.1. Screening assay

A screening assay was performed to confirm isolated peaks of some medicinal toxicants. Sample preparation of the postmortem blood and screening condition were carried out as described below. Acetonitrile deproteinization was performed for the pretreatment of blood. In brief, 100 μL of whole blood was diluted with 200 μL with acetonitrile. The mixture was centrifuged at 10,000×*g* for 10 min at 4 °C. Five microliters of the supernatant was injected into the UPLC–MS system to measure retention time and mass. UPLC was performed with an Acquity® UPLC system (Nihon Waters K.K., Tokyo, Japan) and mass spectrometry was performed with a ZQ® MS system (Nihon Waters K.K.). UPLC-grade ammonium formate, formic acid, acetonitrile, and extra pure water were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Analysis conditions for UPLC were as follows: The separation was carried out using an ACQUITY UPLC HSS C18 Column (1.8 μm, 2.1 mm × 150 mm). The mobile phase consisted of mobile phase A (5 mmol/L ammonium formate and 0.1% formic acid) and mobile phase B (acetonitrile and 0.1% formic acid). The following gradient was used: 13% B (0–0.5 min), 50% B (0.5–10.0 min), 95% B (10.0–10.75 min), 95% B (10.75–12.25 min), 13% B (12.25–12.50 min), 13% B (12.50–15.00 min). The solvent flow rate was 0.4 mL/min, analysis time was 15 min, and column temperature was 50 °C. Analysis conditions for MS were as follows: molecular weight range (80–650 *m*/*z*), analysis time (0–15 min), ion scan mode (electrospray ionization (−) cone voltage 20 V, electrospray ionization (+) cone voltage 20, 35, 50, 65, 80, and 95 V), capillary voltage (3.0 kV), source temperature (120 °C), desolvation temperature (400 °C), desolvation gas flow (800 L/h), and cone gas flow rate (100 L/h). Evaluation was performed using identification software (Chroma Lynx XS®, Nihon Waters K.K.).

2.2.2. SIR mode assay of drospirenone

A SIR mode assay was performed to confirm DRSP which molecular weight is 366.49. Sample preparation of the blood was carried out as described below. Acetonitrile deproteinization was performed for the pretreatment of blood. In brief, each of 100 μL of the postmortem blood and 100 μL of the blood from a healthy volunteer added 50 ng of DRSP (500 ng/mL) was diluted with 200 μL with acetonitrile. The mixture was centrifuged at 10,000×*g* for 10 min at 4 °C. Five microliters of the supernatant was injected into the UPLC–MS system to measure retention time and mass. The equipment for UPLC–MS system was performed with the same as a screening assay. Standard DRSP was obtained from Sigma–Aldrich Co. LLC. (Tokyo, Japan). Analysis conditions for UPLC were the same above. Analysis conditions for MS were as follows: molecular weight range (367.3 *m*/*z*), analysis time (0–15 min), ion scan mode (electrospray ionization (+) cone voltage 35 V), capillary voltage (3.0 kV), source temperature (120 °C), desolvation temperature (400 °C), desolvation gas flow (800 L/h), and cone gas flow rate (100 L/h). Evaluation was performed using identification software (Chroma Lynx XS®, Nihon Waters K.K.).

2.2.3. Quantitative analysis of drospirenone

A quantitative analysis of drospirenone was carried out by UPLC–MS and an application manager (QuanLynx®, Nihon Waters K.K.) as follows. To obtain a calibration curve, blood was collected from a healthy volunteer, and 900 μL whole blood was mixed vigorously with 100 μL of 100 μg/mL DRSP/acetonitrile solution to make a final DRSP concentration of 10 μg/mL, and the mixture was further diluted to make concentration gradients of 1000, 500, 100, 50, and 10 ng/mL. A liquid–liquid extraction method was used for pretreatment, in which 100 μL of the sample was mixed with 200 μL of 75:25 ethyl acetate: hexane solution. After centrifugation at 10,000×*g* for 10 min at 4 °C, the supernatant was collected, dried, and resuspended with 100 μL acetonitrile. UPLC–MS was used to analyze 5 μL of each sample to determine the retention time, mass, and peak area.

Ultra-performance liquid chromatography was performed with the Acquity® UPLC system and mass spectrometry was performed with the ZQ® MS system. UPLC-grade ammonium formate, formic acid, acetonitrile, and extra pure water were purchased from Wako Pure Chemical Industries, Ltd. (Osaka,