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Mifepristone (RU 486) induces vasodilation and inhibits platelet aggregation: nongenomic and genomic action to cause hemorrhage

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Abstract

Background: The regimen mifepristone/misoprostol is an established and highly effective method for early termination of pregnancy. However, its side effects such as a significantly long bleeding time and hemorrhage have been scantly studied.

Study Design: Human umbilical artery (HUA) from pregnant women undergoing elective cesarean section at term and rat thoracic aorta (RTA) were isometrically recorded. The vasorelaxing effect of mifepristone was analyzed on the contractile responses induced by KCl or serotonin (5-HT); moreover, the potential response of mifepristone on adenosine diphosphate (ADP)-induced human platelet aggregation was also evaluated.

Results: This study describes that mifepristone elicits (1) rapid and reversible vasorelaxation on KCl- or 5-HT-induced contraction in HUA and RTA with and without endothelium and (2) immediate prevention of ADP-induced human platelet aggregation.

Conclusions: These effects seem to be responsible for increased and prolonged hemorrhage. Since mifepristone-prevented platelet aggregation was observed in the anucleate platelets, and mifepristone-induced vasorelaxation remained unaffected in de-endothelized tissues, by inhibitors of transcription and translation and a nitric oxide (NO) synthase inhibitor, a nongenomic endothelium- and NO-independent mechanism was revealed. Additionally, the results indicated a blockade of voltage- and receptor-operated calcium channels. The antiglucocorticoid genomic action of mifepristone, by inducing an excess of NO, may also contribute to exacerbated hemorrhage.

Keywords: RU 486; Mifepristone; Medical abortion; Vasodilation; Platelet aggregation; Nongenomic effects

1. Introduction

The synthetic steroid mifepristone (RU 486; trade name Mifeprex), with antiprogesterone and antiglucocorticoid activities, has been approved in several countries for use in four indications: (i) early termination of pregnancy (TOP), (ii) cervical dilatation prior to surgical TOP, (iii) preparation for prostaglandin-induced TOP during the second-trimester and (iv) expulsion of dead fetus during the third-trimester (for review, see Ref. [1]). Regarding the first indication, a number of experimental studies have clearly documented that use of the combined treatment with mifepristone and a prostaglandin analogue (gemeprost or misoprostol) is highly effective for TOP [2–7]. At present, since 2000, mifepristone/misoprostol is the only approved regimen by the Food and Drug Administration (FDA). This medical abortion regimen has since been widely used, but the action of its side effects has not been adequately studied.

In this respect, mifepristone, used as abortifacient, has been linked with serious adverse reactions such as bacterial infections, sepsis and severe bleeding. Reports also observe ectopic pregnancies that have ruptured, fetal malformations that appeared after failed abortion attempts, unsuccessful TOP and several deaths that may occur after use. This information was reviewed from the adverse events data gathered through the FDA's Adverse Event Reporting System [8]. On this basis, and reinforced with subsequent reports [9–11], it has been advised that the two most serious adverse events are hemorrhage and infections which are the leading causes of mifepristone-related morbidity and mortality.

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With regard to the hemorrhage in mifepristone abortion, FDA-approved information for patients (Medication Guide) explains that mifepristone treatment may cause vaginal bleeding. In some cases vaginal bleeding can be very heavy and will need to be stopped by a surgical procedure. It is important to emphasize that the hemorrhages following mifepristone abortion appear to be more severe than those found in surgical or spontaneous abortions [12]; thus, it has been pondered that the antiglucocorticoid action of mifepristone may result in an excess of nitric oxide (NO), which was hypothesized to be the cause of excessive hemorrhage seen in mifepristone abortions [9]. However, notably, no direct experimental studies have been conducted on vascular smooth muscle (blood vessels) reactivity and regulation of homeostasis.

Very recently, our group has shown that mifepristone alone is capable of relaxing the contractile activity of uterine smooth muscle through nongenomic mechanisms involving the blockade of voltage- and receptor-operated calcium channels, which reveals that this antiprogestin may induce a nongenomic antiuterotonic effect prior to its genomic antiprogesterone action [13]. These data raise the possibility that mifepristone may be effective in inducing relaxation in vascular smooth muscle. Therefore, the present study set out to investigate the potential vasorelaxing effect of mifepristone on the vascular reactivity of isolated human umbilical arteries (HUA) at the end of pregnancy. Accordingly, we have investigated the role of vascular endothelium on this process; hence, the rat thoracic aorta (RTA), which can be considered as typical of the type of blood vessel used for previous studies of endothelial and vascular smooth muscle interactions, was also used in most protocols for comparative purposes. In order to shed further light on the process(es) of mifepristone-caused hemorrhage, additional experiments were performed to explore whether mifepristone may affect platelet aggregation. The overall results allowed for understanding of the possible involvement of genomic and nongenomic pathways in the mode action causing hemorrhage for this synthetic steroid, mifepristone.

2. Materials and methods

This study, approved by the Institutional Review Board for Human Research Committee of the Institute for Biomedical Research, National Autonomous University of Mexico, was performed in accordance with The Declaration of Helsinki. Likewise, the animal protocols were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.1. Samples and tension measurement in isolated blood vessels

Umbilical cords were obtained, with written consent, from term healthy pregnancies (38–40 weeks gestation) from women undergoing elective (nonlabor) cesarean section. Segments of umbilical cord (~10 cm in length) were cut and placed in ice cold low-Ca^{2+} Ringer of the following composition (mM): NaCl (110), KCl (5), $CaCl_2$ (0.16), $MgCl_2$ (2), $NaHCO_3$ (10), NaH_2PO_4 (0.5), glucose (10) and EDTA (0.49), resulting in a pH of 6.9. The HUA was dissected from the cord, and the adherent Wharton's jelly was removed. The clean HUA was cut into rings 1 cm in length and stored at 4°C. Experiments were performed 24 and 48 h after HUA rings had been refrigerated. The HUA rings were transferred to Krebs-Henseleit bicarbonate solution (KHS) with the following composition (mM): $NaHCO_3$ (24.9), NaCl (119.5), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $CaCl_2$ (2.5) and glucose (12.0); this solution was gassed continuously with 95% O_2 in 5% CO_2 to maintain pH 7.4 and constant temperature (37°C). On the other hand, the endothelium has an atypical function in the HUA since (i) acetylcholine-induced endothelium-dependent relaxation is weak as compared with other blood vessels [14], and (ii) the biosynthesis of endothelial nitric oxide (NO) is absent [15]. On this basis, we did not remove HUA endothelium, but in some experiments we decided to analyze the role of NO on mifepristone-induced vasorelaxation by using an inhibitor of NO synthase.

Adult male Wistar rats weighing 230–250 g were also used. The rats were killed by decapitation, and their descending thoracic aorta was removed and placed in a KHS with the same composition described above. The midthoracic region was cleaned of fat, blood and connective tissue and cut into sectional rings of 1 cm in length. To assess the role of the endothelium in the vascular response to mifepristone, during preparation of the rings, care was taken to avoid stretching the tissue or touching the luminal surface to preserve endothelial integrity (preparation with endothelium), and some aortas were denuded before mounting by gently rubbing the luminal surface with a plaited nylon string (preparations without endothelium). Before each experiment, endothelial cell integrity was determined in each preparation.

The HUA and RTA rings were mounted in 10-mL tissue chambers containing KHS at 37°C continuously gassed with O_2/CO_2 95:5. The resting tension was adjusted to 2 g for HUA and to 1 g for RTA. Contractions were recorded with an isometric force transducer (FTO3C; Grass Instrument, Quincy, MA, USA) connected to the computerized data acquisition and analysis system (MP150; Biopac Systems, CA, USA). Vascular rings from the HUA were equilibrated for 2 h and those from the RTA for 1 h before conducting the experiments.

The absence of endothelium in rubbed RTA was determined when 20 μM acetylcholine (ACh) failed to induce endothelium-dependent vascular relaxation on 0.3 μM noradrenaline (NA)-induced contraction. RTA rings were then washed with KHS to re-equilibrate for 60 min. The vasocontractile response to KCl (40 mM in HUA or 60 mM in RTA) was induced after replacing KHS with an equimolar substitution of KCl and NaCl. KCl caused a tonic contraction, in both HUA and RTA rings, which was