




RESEARCH

Steroid profile in patients with breast cancer and in mice treated with mifepristone

Andres Elía^{1,*}, Leo Saldain^{1,*}, Silvia Lovisi², Paula Martínez Vazquez², Javier Burruchaga², Caroline A Lamb¹, Isabel Alicia Lüthy¹, Federico Diez³, Natalie Z M Homer³, Ruth Andrew³, Paola Rojas¹ and Claudia Lanari¹

¹Instituto de Biología y Medicina Experimental (IBYME), CONICET, Argentina

²Hospital de Agudos “Magdalena V de Martínez”, General Pacheco, Argentina

³University/BHF Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, UK

Correspondence should be addressed to N Z M Homer: n.z.m.homer@ed.ac.uk

* (A Elía and L Saldain contributed equally to this work)

Abstract

Progesterone receptors (PRs) are biomarkers used as prognostic and predictive factors in breast cancer, but they are still not used as therapeutic targets. We have proposed that the ratio between PR isoforms A and B (PRA and PRB) predicts antiprogestin responsiveness. The MIPRA trial confirmed the benefit of 200 mg mifepristone, administered to patients with tumors with a high PRA/PRB ratio, but dose-ranging has not been conducted. The aim of this study was to establish the plasma mifepristone levels of patients from the MIPRA trial, along with the resultant steroid profiles, and compare these with those observed in mifepristone-treated mice using therapeutic schemes able to induce the regression of experimental mammary carcinomas with high PRA/PRB ratios: 6 mg pellets implanted subcutaneously, or daily doses of 12 mg/kg body weight. The plasma levels of mifepristone and other 19 plasma steroids were measured by liquid chromatography–tandem mass spectrometry. In mifepristone-treated mice, plasma levels were lower than those registered in mifepristone-treated patients (i.e. day 7 after treatment initiation, pellet-treated mice: 8.4 ± 3.9 ng/mL; mifepristone-treated patients: 300.3 ± 31.7 ng/mL (mean \pm s.d.; $P < 0.001$)). The increase in corticoid related steroids observed in patients was not observed in mifepristone-treated mice. The increase in progesterone levels was the most significant side effect detected in mifepristone-treated mice after 14 or 21 days of treatment, probably due to an ovarian compensatory effect not observed in postmenopausal patients. We conclude that in future clinical trials using mifepristone, the possibility of lowering the standard daily dose of 200 mg should be considered.

Keywords: mifepristone; progesterone receptor isoforms; breast cancer treatment; window of opportunity trial; steroid hormone levels; plasma; mice; antiprogestins

Introduction

The idea that targeting the progesterone receptor (PR) in breast cancer could be a therapeutic approach has been considered for decades (reviewed in [Abulafia et al. \(1999\)](#), [Lanari et al. \(2012\)](#), [Giulianelli et al.](#)

[\(2021\)](#)). Moreover, attempts had been performed using progestins such as medroxyprogesterone acetate (MPA) which was given in doses of up to 1 g daily ([Pannuti et al. 1988](#)) or antiprogestins such as mifepristone ([Perrault](#)

et al. 1996), onapristone (Robertson *et al.* 1999, Cottu *et al.* 2018), and, more recently, telapristone acetate (Lee *et al.* 2019). Although partial responses were observed in several patients, the results obtained were less encouraging than originally expected (Lanari *et al.* 2012).

The MIPRA trial was launched following the hypothesis that the PR isoform ratio is crucial to predict the antiprogestin responsiveness of luminal breast carcinomas. This concept was based on previous basic research, mainly reported by the Edwards (Beck *et al.* 1993) and Horwitz (Tung *et al.* 1993) laboratories, who demonstrated that mifepristone acted as a progesterone agonist regulating gene expression in cells expressing PR isoform B (PRB). Along the same line, we have shown that antiprogestin-responsive tumors from the murine MPA-induced breast cancer model had higher levels of PR isoform A (PRA) than PRB (Helguero *et al.* 2003), and when they acquired antiprogestin resistance there was a change in the PR isoform ratio toward B and thus a potential switch from mifepristone acting as an antagonist to an agonist (Helguero *et al.* 2003, Wargon *et al.* 2009, 2011, 2015). In constitutive resistant tumors this change in ratio was due to selective PRA promoter methylation (Wargon *et al.* 2011), and in acquired resistant tumors the mechanism is still under investigation. Preclinical studies using *ex vivo* human breast tissue cultures and cell derived xenograft studies confirmed the murine data (Rojas *et al.* 2017). Encouragingly, results of the MIPRA window of opportunity study revealed a reduction of the proliferation index in 70% of tumors in patients with higher levels of PRA than PRB receiving 200 mg of mifepristone tablets *per os* for 14 days (Elía *et al.* 2022). This dose was selected since it was already proven to be effective and safe for the treatment of meningiomas over long periods of time (Grunberg *et al.* 2006, Ji *et al.* 2015, Karena *et al.* 2022).

Many questions arose after analyzing the MIPRA data, one being whether the dose selected was the most appropriate to treat breast cancer patients. Although mild side effects were observed in treated patients (Elía *et al.* 2022), this agent would be potentially administered together with antiestrogenic compounds, and lower doses may be safer for the patients in the long term, if still efficacious. To address this issue, we decided to compare the mifepristone plasma concentrations of mice receiving mifepristone schedules able to induce complete mammary tumor regression with those detected in mifepristone-treated patients, and in addition, to compare the pharmacodynamic changes in the concentration of the steroids related to the biosynthesis of corticoid/sex steroids. These data might then inform researchers whether lower doses could be assessed in the clinical setting.

Materials and methods

Study design

The MIPRA study was an open-label, interventional, prospective, single-arm clinical trial (ClinicalTrials.gov identifier: NCT02651844). The main goal was to evaluate the effect of mifepristone in postmenopausal breast cancer patients with higher levels of PRA than PRB, naïve from any other treatment. The primary outcome was to compare the proliferation index pre-treatment and post treatment. The trial included patients who spontaneously attended the Magdalena V. de Martinez Hospital at General Pacheco, Buenos Aires. Eligibility criteria included (i) postmenopausal status more than 1 year after the last menses, (ii) tumors larger than 15 mm, (iii) PRA/PRB ratios higher than 1.5 determined by Western blot, (iv) PR total levels $\geq 50\%$ evaluated by immunohistochemistry, (v) World Health Organization condition of 1 with the adequate function of organs and systems: absolute neutrophil count $1500/\text{mL}$; platelets $\geq 100,000/\text{mL}$; hemoglobin $\geq 10 \text{ g/dL}$; CD4 count ≥ 400 ; creatinine $< 1.5 \text{ mg/dL}$; total bilirubin, aspartate aminotransferase, and alanine aminotransferase $< 1.5 \text{ U/L} \times$ upper limit of institutional normal. Patients were excluded if they (i) received any other treatment for cancer, (ii) had hepatitis or human immunodeficiency virus infection, (iii) had cognitive alterations that limited their understanding of the protocol, (iv) experienced a prolonged QT/QTc basal interval, or (v) had asthma or other autoimmune diseases. Twenty patients that met these criteria were treated with daily doses of 200 mg mifepristone administered as tablets (Abortab, Pharma-web; Canada) *per os* during the morning for 14 days.

Studies with patients' plasma

Plasma samples were obtained before ($n=14$) or after 7 ($n=16$) or 14 ($n=13$) days of mifepristone treatment (Fig. 1, top). In all cases, the blood was obtained prior to the mifepristone tablet administration. Nine of these patients completed the entire schedule, whereas in others, one of the plasma samples was missing. Plasma samples were collected in Vacutainer tubes and kept at -20 or -80°C until the study was ended. Aliquots of $200 \mu\text{L}$ were sent to the Mass spectrometry core of the Edinburgh Clinical Research Facility, University of Edinburgh. The LC system was a Water I Class, mobile phase A of 50 mM ammonium fluoride and mobile phase B of 50 mM ammonium fluoride in methanol: Column Kinetex C18 ($150 \times 2.1 \text{ mm}$; $2.6 \mu\text{m}$): Mass Spect: Qtrap 6500+. Mifepristone together with other 19 steroids was measured using a supported liquid extraction method and LC-MS/MS (Denham 2020). The lowest and upper levels of detection, as well

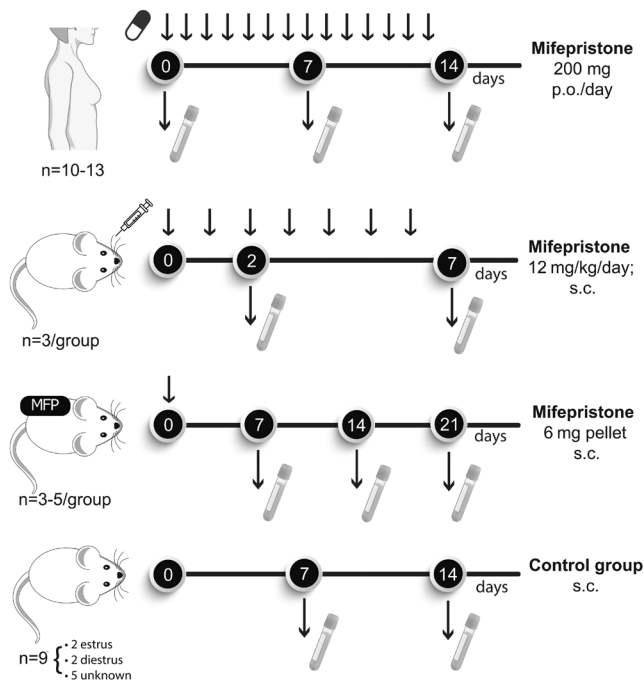


Figure 1

Scheme illustrating mifepristone administration and plasma collection in patients from the MIPRA trial and in mifepristone-treated mice.

as the normal plasma concentration of each steroid, are shown in Supplementary Table 1 (see section on [supplementary materials](#) given at the end of this article).

Mouse studies

Animals

Two-month-old virgin female BALB/c mice (IBYME Animal Facility) were used. All animals were fed *ad libitum* and kept in air-conditioned room at $20 \pm 2^\circ\text{C}$ with a 12-h light–12-h darkness cycle period.

Murine mammary carcinomas

Murine mammary carcinomas from the MPA-induced breast cancer model were used (Lanari *et al.* 2009). The murine mammary carcinoma 59-2-HI, which expresses high estrogen receptor alpha (ER) and PR levels, regresses completely when a 6 mg mifepristone pellet is implanted subcutaneously (Vanzulli *et al.* 2002). Tumors were transplanted into BALB/c mice by trocar and, when they reached a size of 5 mm in the long axis, an MFP pellet (6 mg) was implanted subcutaneously as described (Sahores *et al.* 2013). Tumors were measured twice a week with a Vernier caliper to assess the functionality of pellets.

Mouse plasma

Adult female mice were implanted with mifepristone (6 mg) pellets, or were sham-treated with Silastic pellets without hormones. Mice were bled by cardiac puncture 1 ($n=5$), 2 ($n=3$), or 3 ($n=3$) weeks after pellet implantation, and plasma samples prepared and stored at -80°C . Control mice were bled 1 ($n=2$) or 2 ($n=3$) weeks after empty pellet implantation (Fig. 1, bottom). In addition, further mice were treated with daily injections of mifepristone (12 mg/kg body weight) subcutaneously, a treatment protocol that also induces the regression of this tumor variant (Wargon *et al.* 2009). Mice were bled 48 h or 7 days after treatment initiation ($n=3$ /group). In both dosing scenarios, mice were bled 24 h after drug inoculation. Plasma from three untreated mice were used as controls. From the nine control mice, two were in estrus and two in diestrus. In the other 5 control mice, the estrous cycle was not determined. Samples were sent to the Mass spectrometry Core of the Edinburgh Clinical Research Facility, as explained earlier, to measure the levels of mifepristone and other plasma steroids.

Statistical analysis

Nonmatched plasma levels were compared using nonparametric Kruskal–Wallis tests and Dunn's multicomparison *post hoc* tests. For comparison of plasma levels from the same individual, data were analyzed with nonparametric test for matched data (Friedman test). Tumor growth curves were compared using two-way ANOVA and Tukey *post t*-tests.

Ethics statement

A written informed consent was obtained from all patients. This MIPRA trial was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Boards of the hospital and IBYME (2012-026). Mouse experiments were approved by the IBYME-CONICET Institutional Animal Care and Use Committee authorities (032-2018) and complied with standards of animal ethics. The approval included the experiments reported herein and others that were not included in this article.

Results

Mifepristone levels in plasma from mifepristone-treated mice as compared with those of mifepristone-treated patients

Mifepristone administered as pellets (6 mg; Fig. 2A) or as subcutaneous daily doses of 12 mg/kg body weight (Wargon *et al.* 2009) induced complete regression of ER+ PR+ mammary carcinomas with higher levels

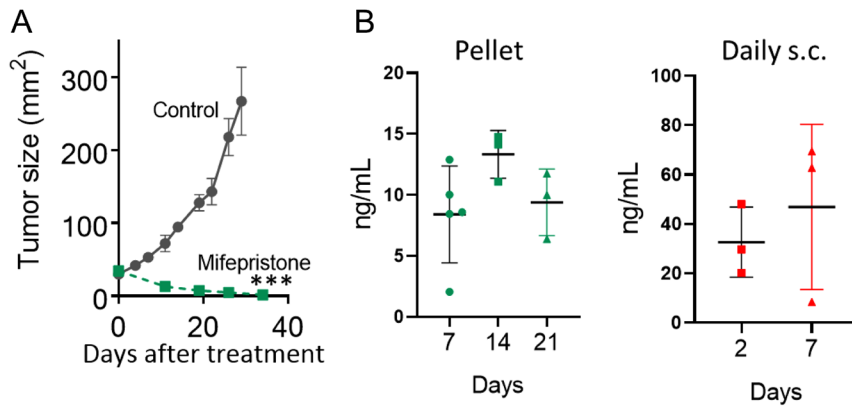


Figure 2

Mifepristone plasma levels capable of inducing complete regression of a mouse mammary carcinoma with PRA levels higher than PRB. (A) Tumor growth curves illustrating 59-2-HI tumor regression after administering a 6 mg mifepristone pellet subcutaneously at day 0; (n = 5); (B) Mifepristone plasma levels (mean ± s.d.) after 7, 14, or 21 days after pellet implantation (left) or after 48 h or 7 days of daily doses of 12 mg/kg body weight (right). A full color version of this figure is available at <https://doi.org/10.1530/ERC-23-0238>.

of PRA than PRB (Lanari et al. 2009). Thus, our first goal was to compare the mifepristone plasma levels in these tumor models in which we already know that PR are involved in their growth (Lamb et al. 1999, 2005) with those observed in mifepristone-treated patients.

As observed in Fig. 2B left, plasma mifepristone levels remained almost constant during 21 days after pellet implantation. Plasma levels were more heterogeneous following daily injections (Fig. 2B right; i.e. 7 days pellet: (mean ± s.d.) 8.4 ± 3.9 ng/mL, 21.5 ± 9.1 nmol/L (n=5); 7 days daily doses: (mean ± s.d.) 46.9 ± 33.4 ng/mL, 109 ± 77 nmol/L (n=3)). Despite these differences, both doses induced complete tumor regression. In patients, significant differences were not observed between mifepristone levels after 1 or 2 weeks of treatment (day 7 (mean ± s.d.): 300.3 ± 31.7 ng/mL (690 ± 72.9

nmol/L); day 14: 320 ± 54.3 ng/mL (745 ± 126.4 nmol/L; n=11); (Elia et al. 2022)), and they were more than twenty times higher than those observed in the animal model using mifepristone pellets (P < 0.001), suggesting that the effect of lower mifepristone doses in breast cancer patients with higher levels of PRA than PRB should be tested.

Pharmacodynamic responses: steroid plasma levels in breast cancer patients or mice treated with mifepristone

Since mifepristone at high concentrations exerts antigluccorticoid (Gaillard et al. 1984) and antiandrogenic (Song et al. 2004) effects, we decided to compare the plasma steroid profile of mifepristone-

Table 1 Steroid hormones in postmenopausal breast cancer patients untreated or treated with mifepristone for 7 or 14 days.

| Steroid | Pre-treatment (nmol/L) n = 14 | | Mifepristone, day 7 (nmol/L) n = 16 | | Mifepristone, day 14 (nmol/L) n = 13 | | P ^a |
|--------------------------|-------------------------------|--------------------|-------------------------------------|--------------------|--------------------------------------|--------------------|----------------|
| | Median (range) | $\bar{X} \pm S.D.$ | Median (range) | $\bar{X} \pm S.D.$ | Median (range) | $\bar{X} \pm S.D.$ | |
| Pregnenolone | 0.62 (0.01–1.5) | 0.7 ± 0.5 | 0.86 (0.09–73.9) | 5.7 ± 18.2 | 1.6 (0.23–6) | 2.2 ± 1.8 | P < 0.05 |
| 11-dehydrocorticosterone | 2.4 (1.2–5.3) | 2.6 ± 1 | 3.7 (1.9–5.5) | 3.7 ± 1.1 | 3.9 (2.3–5.5) | 3.8 ± 0.9 | P < 0.01 |
| 11-deoxycorticosterone | 0.08 (0.03–0.2) | 0.03 ± 0.06 | 0.2 (0.04–0.8) | 0.2 ± 0.2 | 0.2 (0.07–0.4) | 0.2 ± 0.1 | P < 0.01 |
| 11-deoxycortisol | 1.1 (0.5–1.8) | 1.2 ± 0.5 | 3.2 (1–9) | 4.0 ± 2.5 | 3.2 (1.5–5.8) | 3.5 ± 1.2 | P < 0.001 |
| 17-hydroxyprogesterone | 1.01 (0.5–37.5) | 3.6 ± 9.7 | 3.2 (1.3–54.6) | 7.3 ± 13 | 3.7 (1.6–9) | 4.3 ± 2.2 | P < 0.001 |
| 20beta-dihydrocortisol | 3 (2–5.7) | 3.4 ± 1.1 | 5.2 (2.2–10.3) | 5.8 ± 2.6 | 6.8 (3.4–24.5) | 9.1 ± 6.1 | P < 0.001 |
| 21-deoxycortisol | 0.08 (0.02–10.4) | 0.8 ± 2.7 | 0.6 (0.1–8.6) | 1.2 ± 2 | 0.6 (0.19–4.7) | 1.2 ± 1.4 | P < 0.001 |
| Aldosterone | 0.19 (0.02–0.5) | 0.19 ± 0.1 | 0.2 (0.05–0.4) | 0.2 ± 0.1 | 0.1 (0.02–0.3) | 0.1 ± 0.09 | ns |
| Androstenedione | 2.2 (1.2–5.3) | 2.2 ± 1.1 | 3.6 (1.5–10.6) | 4.5 ± 2.7 | 4.5 (2.6–6.7) | 4.1 ± 1.4 | P < 0.001 |
| Corticosterone | 9.8 (4.3–30.8) | 11.9 ± 7.4 | 27.1 (6.6–49.2) | 25.7 ± 14.1 | 21.2 (8.2–52.4) | 21.7 ± 11.2 | P < 0.01 |
| Cortisol | 447 (268–530) | 425 ± 89 | 730 (413–1014) | 712 ± 199 | 747 (493–1215) | 780 ± 176 | P < 0.001 |
| Cortisone | 65 (47–96) | 66 ± 13 | 74 (51–130) | 76 ± 17 | 80 (70–82) | 82 ± 10 | P < 0.01 |
| Dihydroepiandrosterone | 10.6 (4–21) | 12 ± 5 | 19 (8–35) | 20 ± 8 | 22 (7–51) | 24 ± 13 | P < 0.01 |
| Dihydrotestosterone | 0.26 (0.16–0.46) | 0.3 ± 0.09 | 0.5 (0.22–0.95) | 0.5 ± 0.2 | 0.62 (0.2–1) | 0.6 ± 0.2 | P < 0.001 |
| Estradiol | 0.05 (0.01–0.09) | 0.05 ± 0.02 | 0.06 (0.03–0.4) | 0.08 ± 0.07 | 0.08 (0.03–0.1) | 0.07 ± 0.02 | P < 0.05 |
| Estrone | 0.14 (0.06–0.24) | 0.15 ± 0.05 | 0.2 (0.09–0.5) | 0.2 ± 0.1 | 0.28 (0.1–0.51) | 0.28 ± 0.1 | P < 0.01 |
| Progesterone | 0.13 (0.09–1.3) | 0.3 ± 0.4 | 0.3 (0.07–2.1) | 0.6 ± 0.6 | 0.4 (0.1–1) | 0.5 ± 0.3 | ns |
| Testosterone | 0.56 (0.34–2) | 0.7 ± 0.4 | 1 (0.5–2.8) | 1.2 ± 0.7 | 1 (0.6–2.4) | 1.2 ± 0.5 | P < 0.01 |

^aKruskal–Wallis test.

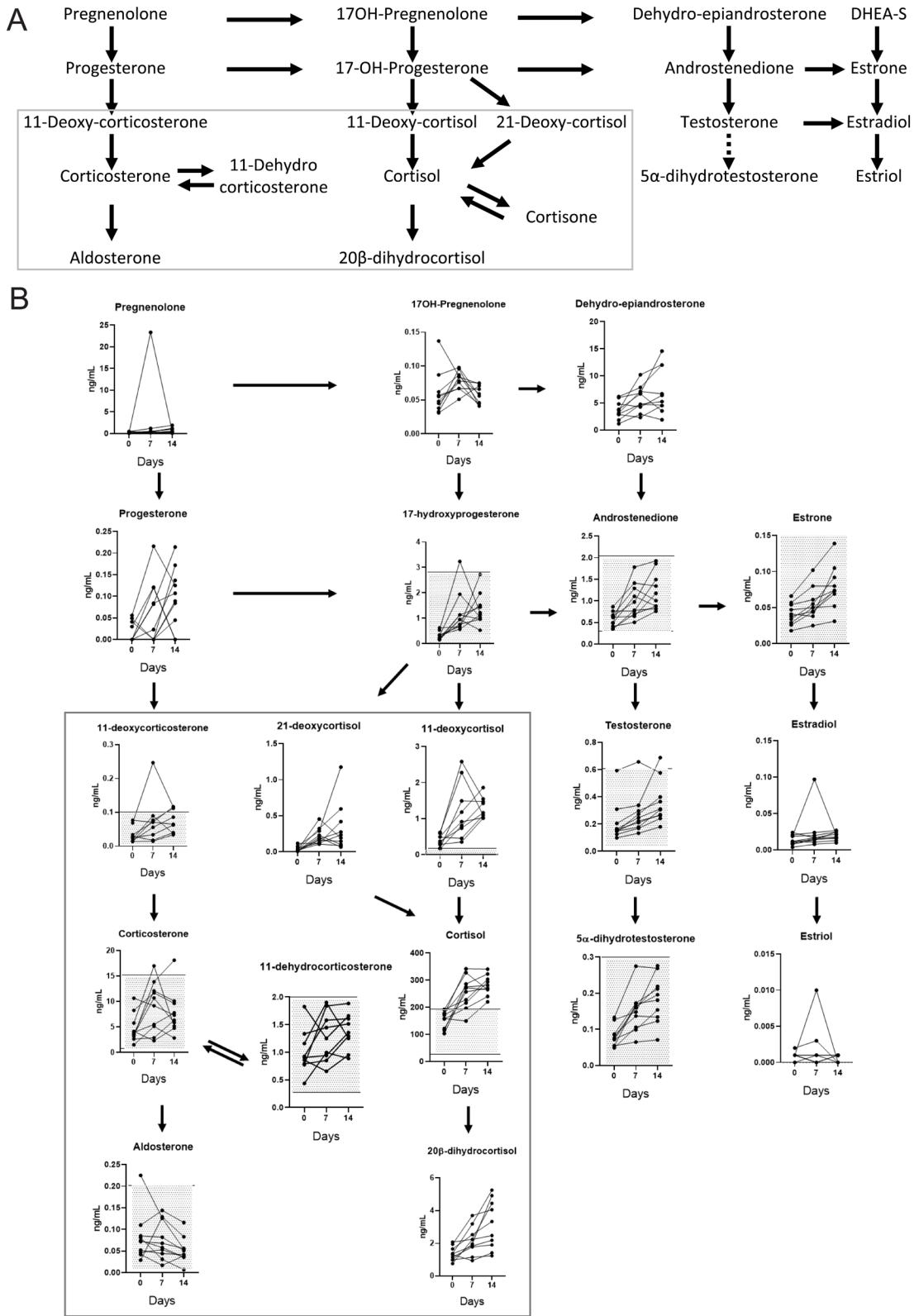


Figure 3

Changes in steroid hormone levels in postmenopausal breast cancer patients after mifepristone treatment. (A) Human steroidogenesis pathway. (B) Profile of 19 steroid hormones evaluated in plasma by liquid extraction and LC-MS/MS before and after 7 or 14 days of mifepristone treatment. Values that fall in dotted areas are within normal levels detected in pre- or postmenopausal women. In all cases there was a significant change after treatment. Data were analyzed using a nonparametric analysis for matched samples (Friedman test). In most cases, except for cortisol, the changes were within physiological levels. The data of nine patients are shown. The graphs are arranged according to the pathways shown in A.

treated breast cancer patients with that of mifepristone-treated mice.

The plasma levels of steroids related to the corticoid, androgenic, and estrogen biosynthesis registered in patients before, and after 7 or 14 days of treatment are shown in Table 1. Significant variations were observed in 17 out of 19 steroids tested; however, most of them remained within physiological levels detected in pre- or postmenopausal women (Supplementary Table 1), except for cortisol, which surpassed the normal levels reaching a mean value of 350 ng/mL at day 14 after treatment, and testosterone, in which 2 values surpassed the upper limit. Changes were not observed in aldosterone and progesterone levels, and those in estrogenic compounds were small, although significant. The individual shifts of each of the 9 patients in which the three measurements were performed is shown in Fig. 3.

We then evaluated the profile of 14 steroids in mifepristone-treated mice after 7, 14, or 21 days of pellet implantation (Table 2). In this case, the only statistically significant change was that of progesterone, in which a late increase after 14 or 21 days of treatment was observed. A similar trend was observed with the precursor pregnenolone and with aldosterone; the values of treated mice vs control mice only showed statistical significance if the values of all treated mice were grouped together ($P < 0.05$). The trend of an increase in the levels of aldosterone could be the product of the apparent decrease in corticosterone levels observed in mifepristone-treated mice. In Fig. 4, we show the levels of the main steroids comparing changes found in mifepristone-treated patients and in treated mice. In this figure, the steroid levels detected in untreated patients or in untreated mice are plotted as square boxes. It can be noticed that whereas in postmenopausal patients the most important potential side effects could be driven by the increase in corticoid-related steroids, this did not occur in mice in which low mifepristone levels were detected. Progesterone-related hormones increased in treated mice, probably counteracting mifepristone's antiprogesterin effects. Importantly, no increases in glucocorticoid steroids were evident in mice treated with mifepristone pellets at doses that may induce tumor regression.

Regarding the intrinsic comparison in the steroid profile between both administration schedules, the only significant difference was the increase in progesterone levels observed after 7 days of treatment with the daily doses (more than 15 times higher than control values). In pellet-treated mice, progesterone levels were similar at day 7 to those observed in control mice and as mentioned before, the increase was observed later, 14 or 21 days after pellet implantation ($P < 0.05$; Fig. 5). The data shown herein suggest that the mifepristone 6 mg handmade pellets may be a better choice than the daily injections.

Table 2 Steroid hormone profile in mice treated with daily doses or with mifepristone pellets.

| Steroid | Control | | 12 mg/kg/day | | | Pellet 6mg | | | P ^a |
|--------------------------|------------------------|------------------|------------------------|------------------|------------------|------------------------|----------------|--|----------------|
| | No drug (n = 9) | | Day 2 (n = 3) | Day 7 (n = 3) | Day 7 (n = 5) | Day 14 (n = 3) | Day 21 (n = 3) | | |
| | Median (range), nmol/L | | Median (range), nmol/L | | | Median (range), nmol/L | | | |
| Mifepristone | - | 69 (47-112) | 146 (19.6-161.4) | 20 (4.8-30) | 32.9 (25.8-34.3) | 23.3 (14.9-27.3) | - | | |
| Pregnenolone | 0.9 (0.09-49.3) | 2.3 (1.4-3.2) | 5.2 (0.04-8.0) | 2.8 (0.13-4.64) | 4.2 (2.2-11.6) | 5.3 (2.9-9.1) | ns | | |
| 11-dehydrocorticosterone | 8.9 (2.0-26.6) | 0.9 (0.6-3.9) | 2.5 (0.2-6.2) | 9.4 (5.7-17) | 5.1 (1.0-6.3) | 4.9 (3.1-8.5) | ns | | |
| 11-deoxycorticosterone | 1.16 (0.3-5.2) | 0.3 (0.2-0.3) | 1.2 (0.7-6) | 2 (0.5-3) | 1.4 (0.3-2.2) | 1.9 (0.5-5.3) | ns | | |
| 17-hydroxyprogesterone | 0.09 (0.03-0.3) | 0.09 (0.03-0.3) | 0.02 (0.01-0.04) | 0.1 (0.07-0.13) | 0.03 (0.02-0.04) | 0.02 (0.02-0.03) | ns | | |
| Aldosterone | 0.4 (0.1-0.8) | 0.05 (0.05-0.05) | 0.2 (0.12-0.24) | 1.2 (0.2-2.5) | 0.03 (0.03-0.04) | 0.2 (0.01-0.2) | ns | | |
| Androstenedione | 0.09 (0.06-1.2) | 0.4 (0.1-0.7) | 0.24 (0.06-0.5) | 0.2 (0.09-0.7) | 0.2 (0.2-0.3) | 0.2 (0.1-0.2) | ns | | |
| Corticosterone | 665 (261-1053) | 160 (111-263) | 293.4 (83-776) | 650 (304-1054) | 333 (60.4-402) | 270 (189-703) | ns | | |
| Estradiol | 0.1 (0.02-0.2) | 0.2 (0.05-0.2) | 0.1 (0.07-0.12) | 0.06 (0.03-0.12) | 0.18 (0.18-0.18) | 0.06 (0.06-0.06) | ns | | |
| Estrone | 0.01 (0-0.02) | 0.01 (0-0.02) | 0.01 (0-0.01) | 0.01 (0-0.01) | 0 (0-0) | 0 (0-0) | ns | | |
| Progesterone | 0.9 (0.3-29.9) | 0.34 (0.3-3.1) | 87 (24-176) | 4.2 (0.2-12.4) | 36.4 (24.3-63) | 33.4 (10-60) | $P < 0.05$ | | |
| Testosterone | 0.06 (0.03-0.9) | 0.35 (0.08-0.4) | 0.19 (0.04-0.43) | 0.1 (0.06-0.51) | 0.15 (0.1-0.2) | 0.2 (0.1-0.3) | ns | | |

^aKruskal-Wallis test.

Discussion

In this study, we have shown that the therapeutic effect of mifepristone on mouse mammary carcinomas occurs at plasma concentrations much lower than those observed in the MIPRA trial, suggesting the possibility to diminish mifepristone doses in

future clinical trials treating breast cancer patients. Lowering these doses could avoid the increase in plasma glucocorticoids levels maintaining the same therapeutic effects.

Mifepristone, in addition to being an antiprogesterin, at higher concentrations behaves as an antiglucocorticoid (Gaillard *et al.* 1984) or as an antiandrogen (Song *et al.*

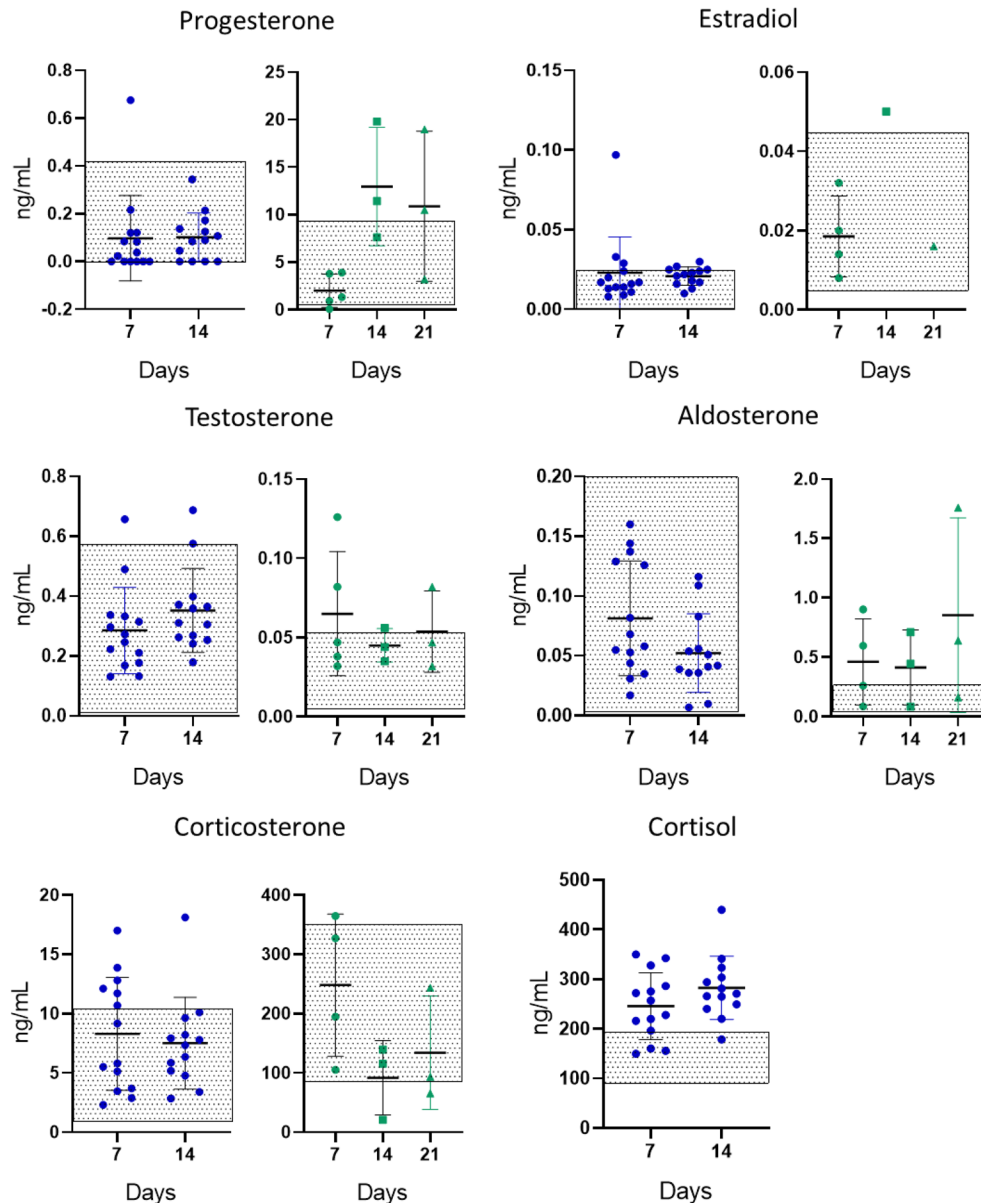


Figure 4

Comparison between the levels of the main steroid hormones in plasma from mifepristone-treated patients vs mifepristone-treated mice. Levels of steroid hormones ($\bar{X} \pm s.d.$) in mifepristone-treated patients after 7 or 14 days of treatment (left graphs in blue) vs. those observed in mice treated with mifepristone pellets for 7, 14, or 21 days (right graphs in green). The values that fall in the dotted areas correspond to values from the untreated patients or the untreated mice respectively. Progesterone levels were higher in mifepristone-treated mice for 14 days compared to those treated for 7 days. Data were analyzed using Kruskal–Wallis test. $*P < 0.05$. The statistical significance of changes observed in patients compared to control values is shown in Table 1. A full color version of this figure is available at <https://doi.org/10.1530/ERC-23-0238>.

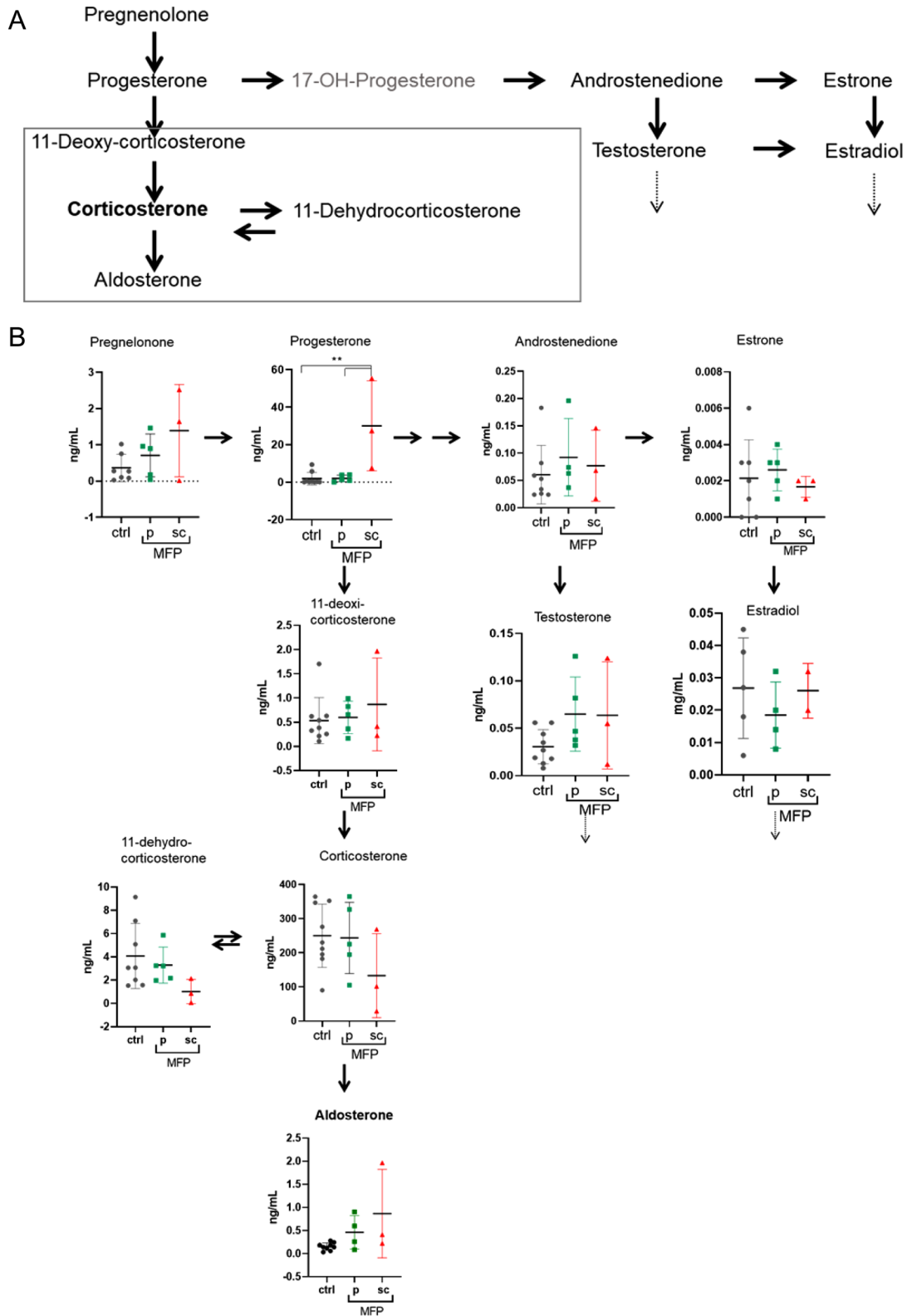


Figure 5

Changes in steroid hormone levels in mifepristone treated mice after 7 days of treatment with daily doses or pellets. (A) Mouse steroidogenic pathways: Scheme illustrating the synthetic pathways of corticoid and sexual related steroids. (B) Profile of different steroid hormones measured by Liquid extraction and LC-MS/MS in plasma from untreated female mice (ctrl) or those treated with mifepristone (MFP) pellets (6 mg) implanted subcutaneously or with daily doses (12 mg/kg body weight) subcutaneously ($\bar{X} \pm s.d.$). Data were analyzed using Kruskal-Wallis test. Progesterone levels were higher in mice treated with daily doses compared with controls or those treated with pellets; $**P < 0.01$. A full color version of this figure is available at <https://doi.org/10.1530/ERC-23-0238>.

2004). In triple-negative breast cancer, mifepristone is also being tested in combination with chemotherapy to increase the efficacy (NCT02788981) (Skor *et al.* 2013). Besides, it has been proposed as a chemotherapeutic agent for advanced PR-negative cancer patients to improve the quality of life (Check *et al.* 2010). In this case, membrane progesterone receptors such as PGRMC1 may be involved, decreasing progesterone-induced blocking factor and thus increasing NK cells (Check & Check 2021). In both cases, the mechanisms proposed are unrelated to the canonical antiprogestin activity of the drug.

The doses used in the MIPRA trial have already been used in long-term clinical studies in which the authors had the intention to minimize the antiglucocorticoid effects (Grunberg *et al.* 2006, Ji *et al.* 2015). In fact for the treatment of Cushing disease, doses of 300–1200 mg/day are currently used (Karena *et al.* 2022). In the first clinical trial in which mifepristone was used to treat metastatic breast cancer patients, the 200 mg dose was selected considering that this dose would be equivalent to the daily 2.5 mg/kg dose administered in preclinical contraception assays in which an antiprogestin effect was assessed (Romieu *et al.* 1987). Furthermore, it was still selected in other trials involving breast cancer patients (Klijn *et al.* 2000). In our short-term study, increases in cortisol levels were observed in a similar range as those encountered by others (Romieu *et al.* 1987, Klijn *et al.* 1989). However, signs of biochemical side effects such as increases in glucose levels were not reflected in the laboratory analysis (Elia *et al.* 2022), suggesting that the cortisol increase after mifepristone treatment may be a compensatory effect that is still not clinically relevant in the 14-day treatment scheme. Interestingly, in patients treated with tamoxifen, an estrogen receptor modulator, the levels of cortisol and corticosterone also significantly increased, with values reaching in some patients near the micromolar range (Baumgart *et al.* 2014).

In humans, the time to maximum plasma concentration after oral administration of mifepristone in doses of 2–600 mg was 1–2 h after ingestion (Heikinheimo *et al.* 1987, Kekkonen *et al.* 1996). Remarkably, the maximum serum concentration was similar among doses between 100 to 800 mg and they remained in the micromolar range persisting up to 20 months after daily treatment (Heikinheimo *et al.* 1997). Only when doses lower than 25 mg were administered, the serum concentrations increased proportionally (Foldesi *et al.* 1996, Kekkonen *et al.* 1996). At higher doses, 94 to 99% of the drug binds to a high affinity binding protein identified as alpha-1-acid glycoprotein (AAG) and to albumin, suggesting that there is a threshold and that if surpassed, the amount of free mifepristone, that exerts the therapeutic effect, will still be the same. Thus, lower mifepristone doses may have a similar therapeutic impact than the higher ones.

The possibility that in mice lower mifepristone levels would be more bioactive because of different AAG and albumin serum concentrations (reviewed in (Heikinheimo 1997, Smith & Waters 2018, Islam *et al.* 2020)) seems unlikely, since the mass spectrometry method used herein measures bound and unbound mifepristone.

The mifepristone doses chosen to treat mouse mammary carcinomas were originally selected from studies carried out using the MXT mouse model, or DMBA-induced mammary carcinomas in rats (Bakker *et al.* 1990, Schneider *et al.* 1990). Then, in our laboratory we designed handmade Silastic pellets (Sahores *et al.* 2013). We have shown that the subcutaneous implantation of 6 mg pellets mimicked the daily dose inducing regression of tumors from the MPA-induced breast cancer model (Wargon *et al.* 2015), some of them being responsive even with pellets of 0.2 mg instead of the standard 6 mg dose (Sequeira *et al.* 2021).

In mice, the active glucocorticoid hormone is corticosterone, and no increases in corticosterone levels were observed in mifepristone-treated mice. In contrast to mifepristone-treated patients, an increase in progesterone-related steroids was observed. This might be a compensatory mechanism present in young female adult mice, not observed in postmenopausal women with nonfunctional ovaries. The increase in aldosterone may be due to an increase in the CYP11B2 enzyme activity that metabolizes corticosterone to aldosterone.

One weakness of this study is that we have compared serum levels of mifepristone between two different species; it is possible that the pharmacokinetics, pharmacodynamics, and possibly also the biological effects differ between both species. However, considering (i) that standard tamoxifen therapy is usually administered as daily tablets of 20 mg for 5–10 years to treat luminal breast cancer patients and the reported plasma tamoxifen levels range from 20 to 307 ng/mL (~545 nmol/L; MacCallum *et al.* (1997), Fotohi *et al.* (2016), Bobin-Dubigeon *et al.* (2019)); (ii) that the antiprogestin telapristone acetate was administered at daily doses of 12 mg and therapeutic effects were observed in patients bearing plasma levels of 147 ± 111 ng/mL (Lee *et al.* 2019); (iii) that mifepristone has a higher affinity for PR than the natural ligands (Kd of mifepristone for the human or mouse PR is $1\text{--}2 \times 10^{-9}$ mol/L (Heikinheimo *et al.* 1987, Montecchia *et al.* 1999)) and mouse tumors regressed completely with plasma levels lower than 20 ng/mL, it seems reasonable to propose the evaluation of lower mifepristone doses to treat luminal breast carcinomas with higher levels of PRA than PRB.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/ERC-23-0238>.

Declaration of interest

Claudia Lanari and Paola Rojas have a patent (WO2013086379A2) regarding the use of antiprogestins in human breast cancer.

Funding

The clinical study was funded by Agencia Nacional de Promoción de Ciencia y Tecnología, Ministerio de Ciencia y Técnica- Argentina, ANAPCYT (PIDC 2012-084) to C. Lanari, the analysis of the human plasma studies was funded by Fundación Sales to C. Lanari, and the analysis of the murine plasma samples was funded by ANAPCYT, PICT 2021-029.

Acknowledgements

We are grateful to Fundación Williams and Gador for their continuous support.

References

- Abulafia O, Triest WE, Adcock JT & Sherer DM 1999 The effect of medroxyprogesterone acetate on angiogenesis in complex endometrial hyperplasia. *Gynecologic Oncology* **72** 193–198. (<https://doi.org/10.1006/gyno.1998.5106>)
- Bakker GH, Setyono-Han B, Portengen H, de Jong FH, Foekens JA & Klijn JG 1990 Treatment of breast cancer with different antiprogestins: preclinical and clinical studies. *Journal of Steroid Biochemistry and Molecular Biology* **37** 789–794. ([https://doi.org/10.1016/0960-0760\(90\)90421-g](https://doi.org/10.1016/0960-0760(90)90421-g))
- Baumgart J, Nilsson K, Stavreus Evers A, Kunovac Kallak T, Kushnir MM, Bergquist J & Sundstrom Poromaa I 2014 Androgen levels during adjuvant endocrine therapy in postmenopausal breast cancer patients. *Climacteric* **17** 48–54. (<https://doi.org/10.3109/13697137.2013.800039>)
- Beck CA, Weigel NL, Moyer ML, Nordeen SK & Edwards DP 1993 The progesterone antagonist RU486 acquires agonist activity upon stimulation of cAMP signaling pathways. *PNAS* **90** 4441–4445. (<https://doi.org/10.1073/pnas.90.10.4441>)
- Bobin-Dubigeon C, Campone M, Rossignol E, Salaun E, Amiard MB & Bard JM 2019 New UPLC-MS/MS assay for the determination of tamoxifen and its metabolites in human plasma, application to patients. *Future Science OA* **5** FSO374. (<https://doi.org/10.2144/fsoa-2018-0113>)
- Check JH & Check D 2021 New Insights as to why progesterone receptor modulators, such as mifepristone, seem to be more effective in treating cancers that are devoid of the classical nuclear progesterone receptor. *Anticancer Research* **41** 5873–5880. (<https://doi.org/10.21873/anticancer.15407>)
- Check JH, Dix E, Cohen R, Check D & Wilson C 2010 Efficacy of the progesterone receptor antagonist mifepristone for palliative therapy of patients with a variety of advanced cancer types. *Anticancer Research* **30** 623–628.
- Cottu PH, Bonnetterre J, Varga A, Campone M, Leary A, Floquet A, Berton-Rigaud D, Sablin MP, Lesoin A, Rezai K, *et al.* 2018 Phase I study of onapristone, a type I antiprogestin, in female patients with previously treated recurrent or metastatic progesterone receptor-expressing cancers. *PLoS One* **13** e0204973. (<https://doi.org/10.1371/journal.pone.0204973>)
- Denham SGJ, G, Kyle CJ, Richardson J, Lee P, Simpson JP, Gifford RM, Andrew R, Reynolds RM & Homer NZ 2020 Automated Supported Liquid Extraction for the analysis of a panel of 12 endogenous steroids in human plasma by LC-MS/MS. *Preprints*. (<https://doi.org/10.20944/preprints202011.0551.v1>)
- Elia A, Saldain L, Vanzulli SI, Helguero LA, Lamb CA, Fabris V, Pataccini G, Martinez-Vazquez P, Burruchaga J, Caillet-Bois I, *et al.* 2022 Beneficial effects of mifepristone treatment in breast cancer patients selected by the progesterone receptor isoform ratio: results from the MIPRA trial. *Clinical Cancer Research* **29** 866–877. (<https://doi.org/10.1158/1078-0432.CCR-22-2060>)
- Foldesi I, Falkay G & Kovacs L 1996 Determination of RU486 (mifepristone) in blood by radioreceptor assay; a pharmacokinetic study. *Contraception* **54** 27–32. ([https://doi.org/10.1016/0010-7824\(96\)00116-3](https://doi.org/10.1016/0010-7824(96)00116-3))
- Fotoohi AK, Karim H, Lafolie P, Pohanka A, Ostervall J, Hatschek T & Vitols S 2016 Pronounced interindividual but not intraindividual variation in tamoxifen and metabolite levels in plasma during adjuvant treatment of women with early breast cancer. *Therapeutic Drug Monitoring* **38** 239–245. (<https://doi.org/10.1097/FTD.0000000000000257>)
- Gaillard RC, Riondel A, Muller AF, Herrmann W & Baulieu EE 1984 RU 486: a steroid with antigluco-corticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day. *PNAS* **81** 3879–3882. (<https://doi.org/10.1073/pnas.81.12.3879>)
- Giulianelli S, Lamb CA & Lanari C 2021 Progesterone receptors in normal breast development and breast cancer. *Essays in Biochemistry* **65** 951–969. (<https://doi.org/10.1042/EBC20200163>)
- Grunberg SM, Weiss MH, Russell CA, Spitz IM, Ahmadi J, Sadun A & Sitruk-Ware R 2006 Long-term administration of mifepristone (RU486): clinical tolerance during extended treatment of meningioma. *Cancer Investigation* **24** 727–733. (<https://doi.org/10.1080/07357900601062339>)
- Heikinheimo O 1997 Clinical pharmacokinetics of mifepristone. *Clinical Pharmacokinetics* **33** 7–17. (<https://doi.org/10.2165/00003088-199733010-00002>)
- Heikinheimo O, Kontula K, Croxatto H, Spitz I, Luukkainen T & Lahteenmaki P 1987 Plasma concentrations and receptor binding of RU 486 and its metabolites in humans. *Journal of Steroid Biochemistry* **26** 279–284. ([https://doi.org/10.1016/0022-4731\(87\)90083-5](https://doi.org/10.1016/0022-4731(87)90083-5))
- Heikinheimo O, Ranta S, Grunberg S, Lahteenmaki P & Spitz IM 1997 Alterations in the pituitary-thyroid and pituitary-adrenal axes--consequences of long-term mifepristone treatment. *Metabolism: Clinical and Experimental* **46** 292–296. ([https://doi.org/10.1016/s0026-0495\(97\)90256-0](https://doi.org/10.1016/s0026-0495(97)90256-0))
- Helguero LA, Viegas M, Asaithamby A, Shyamala G, Lanari C & Molinolo AA 2003 Progesterone receptor expression in medroxyprogesterone acetate-induced murine mammary carcinomas and response to endocrine treatment. *Breast Cancer Research and Treatment* **79** 379–390. (<https://doi.org/10.1023/a:1024029826248>)
- Islam MS, Afrin S, Jones SI & Segars J 2020 Selective progesterone receptor modulators-mechanisms and therapeutic utility. *Endocrine Reviews* **41** bnaa012. (<https://doi.org/10.1210/erndrev/bnaa012>)
- Ji Y, Rankin C, Grunberg S, Sherrod AE, Ahmadi J, Townsend JJ, Feun LG, Fredericks RK, Russell CA, Kabbinnar FF, *et al.* 2015 Double-blind Phase III randomized trial of the antiprogestin agent mifepristone in the treatment of unresectable meningioma: SWOG S9005. *Journal of Clinical Oncology* **33** 4093–4098. (<https://doi.org/10.1200/JCO.2015.61.6490>)
- Karena ZV, Shah H, Vaghela H, Chauhan K, Desai PK & Chitalwala AR 2022 Clinical utility of mifepristone: appraising the expanding horizons. *Cureus* **14** e28318. (<https://doi.org/10.7759/cureus.28318>)
- Kekkonen R, Heikinheimo O, Mandelin E & Lahteenmaki P 1996 Pharmacokinetics of mifepristone after low oral doses. *Contraception* **54** 229–234. ([https://doi.org/10.1016/s0010-7824\(96\)00193-x](https://doi.org/10.1016/s0010-7824(96)00193-x))
- Klijn JG, de Jong FH, Bakker GH, Lamberts SW, Rodenburg CJ & Alexieva-Figusch J 1989 Antiprogestins, a new form of endocrine therapy for human breast cancer. *Cancer Research* **49** 2851–2856.
- Klijn JG, Setyono-Han B & Foekens JA 2000 Progesterone antagonists and progesterone receptor modulators in the treatment of breast

- cancer. *Steroids* **65** 825–830. ([https://doi.org/10.1016/s0039-128x\(00\)00195-1](https://doi.org/10.1016/s0039-128x(00)00195-1))
- Lamb C, Simian M, Molinolo A, Pazos P & Lanari C 1999 Regulation of cell growth of a progestin-dependent murine mammary carcinoma in vitro: progesterone receptor involvement in serum or growth factor-induced cell proliferation. *Journal of Steroid Biochemistry and Molecular Biology* **70** 133–142. ([https://doi.org/10.1016/s0960-0760\(99\)00108-9](https://doi.org/10.1016/s0960-0760(99)00108-9))
- Lamb CA, Helguero LA, Giulianelli S, Soldati R, Vanzulli SI, Molinolo AA & Lanari C 2005 Antisense oligonucleotides targeting the progesterone receptor inhibit hormone-independent breast cancer growth in mice. *Breast Cancer Research* **7** R1111–R1121. (<https://doi.org/10.1186/bcr1345>)
- Lanari C, Lamb CA, Fabris VT, Helguero LA, Soldati R, Bottino MC, Giulianelli S, Cerliani JP, Wargon V & Molinolo A 2009 The MPA mouse breast cancer model: evidence for a role of progesterone receptors in breast cancer. *Endocrine-Related Cancer* **16** 333–350. (<https://doi.org/10.1677/ERC-08-0244>)
- Lanari C, Wargon V, Rojas P & Molinolo AA 2012 Antiprogestins in breast cancer treatment: are we ready? *Endocrine-Related Cancer* **19** R35–R50. (<https://doi.org/10.1530/ERC-11-0378>)
- Lee O, Sullivan ME, Xu Y, Rodgers C, Muzzio M, Helenowski I, Shidfar A, Zeng Z, Singhal H, Jovanovic B, et al. 2019 Selective progesterone receptor modulators in early stage breast cancer: a randomized, placebo-controlled Phase II window of opportunity trial using telapristone acetate. *Clinical Cancer Research* **26** 25–34. (<https://doi.org/10.1158/1078-0432.CCR-19-0443>)
- MacCallum J, Cummings J, Dixon JM & Miller WR 1997 Solid-phase extraction and high-performance liquid chromatographic determination of tamoxifen and its major metabolites in breast tumour tissues. *Journal of Chromatography* **698** 269–275. ([https://doi.org/10.1016/s0378-4347\(97\)00286-7](https://doi.org/10.1016/s0378-4347(97)00286-7))
- Montecchia MF, Lamb C, Molinolo AA, Luthy IA, Pazos P, Charreau E, Vanzulli S & Lanari C 1999 Progesterone receptor involvement in independent tumor growth in MPA-induced murine mammary adenocarcinomas. *Journal of Steroid Biochemistry and Molecular Biology* **68** 11–21. ([https://doi.org/10.1016/s0960-0760\(98\)00166-6](https://doi.org/10.1016/s0960-0760(98)00166-6))
- Pannuti F, Martoni A, Cilenti G, Camaggi CM & Fruet F 1988 Adjuvant therapy for operable breast cancer with medroxyprogesterone acetate alone in postmenopausal patients or in combination with CMF in premenopausal patients. *European Journal of Cancer and Clinical Oncology* **24** 423–429. ([https://doi.org/10.1016/s0277-5379\(98\)90012-8](https://doi.org/10.1016/s0277-5379(98)90012-8))
- Perrault D, Eisenhauer EA, Pritchard KI, Panasci L, Norris B, Vandenberg T & Fisher B 1996 Phase II study of the progesterone antagonist mifepristone in patients with untreated metastatic breast carcinoma: a National Cancer Institute of Canada Clinical Trials Group study. *Journal of Clinical Oncology* **14** 2709–2712. (<https://doi.org/10.1200/JCO.1996.14.10.2709>)
- Robertson JF, Willsher PC, Winterbottom L, Blamey RW & Thorpe S 1999 Onapristone, a progesterone receptor antagonist, as first-line therapy in primary breast cancer. *European Journal of Cancer* **35** 214–218. ([https://doi.org/10.1016/s0959-8049\(98\)00388-8](https://doi.org/10.1016/s0959-8049(98)00388-8))
- Rojas PA, May M, Sequeira GR, Elía A, Alvarez M, Martinez P, Gonzalez P, Hewitt S, He X, Perou CM, et al. 2017 Progesterone receptor isoform ratio: a breast cancer prognostic and predictive factor for antiprogestin responsiveness. *Journal of the National Cancer Institute* **109** djw317. (<https://doi.org/10.1093/jnci/djw317>)
- Romieu G, Maudelonde T, Ulmann A, Pujol H, Grenier J, Cavalié G, Khalaf S & Rochefort H 1987 The antiprogestin RU486 in advanced breast cancer: preliminary clinical trial. *Bulletin du Cancer* **74** 455–461.
- Sahores A, Luque GM, Wargon V, May M, Molinolo A, Becu-Villalobos D, Lanari C & Lamb CA 2013 Novel, low cost, highly effective, handmade steroid pellets for experimental studies. *PLoS One* **8** e64049. (<https://doi.org/10.1371/journal.pone.0064049>)
- Schneider MR, Michna H, Nishino Y & el Etreby MF 1990 Antitumor activity and mechanism of action of different antiprogestins in experimental breast cancer models. *Journal of Steroid Biochemistry and Molecular Biology* **37** 783–787. ([https://doi.org/10.1016/0960-0760\(90\)90420-p](https://doi.org/10.1016/0960-0760(90)90420-p))
- Sequeira GR, Sahores A, Dalotto-Moreno T, Perrotta RM, Pataccini G, Vanzulli SI, Polo ML, Radisky DC, Sartorius CA, Novaro V, et al. 2021 Enhanced antitumor immunity via endocrine therapy prevents mammary tumor relapse and increases immune checkpoint blockade sensitivity. *Cancer Research* **81** 1375–1387. (<https://doi.org/10.1158/0008-5472.CAN-20-1441>)
- Skor MN, Wonder EL, Kocherginsky M, Goyal A, Hall BA, Cai Y & Conzen SD 2013 Glucocorticoid receptor antagonism as a novel therapy for triple-negative breast cancer. *Clinical Cancer Research* **19** 6163–6172. (<https://doi.org/10.1158/1078-0432.CCR-12-3826>)
- Smith SA & Waters NJ 2018 Pharmacokinetic and pharmacodynamic considerations for drugs binding to Alpha-1-acid glycoprotein. *Pharmaceutical Research* **36** 30. (<https://doi.org/10.1007/s11095-018-2551-x>)
- Song LN, Coghlan M & Gelmann EP 2004 Antiandrogen effects of mifepristone on coactivator and corepressor interactions with the androgen receptor. *Molecular Endocrinology* **18** 70–85. (<https://doi.org/10.1210/me.2003-0189>)
- Tung L, Mohamed MK, Hoeffler JP, Takimoto GS & Horwitz KB 1993 Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. *Molecular Endocrinology* **7** 1256–1265. (<https://doi.org/10.1210/mend.7.10.8123133>)
- Vanzulli S, Efeyan A, Benavides F, Helguero LA, Peters G, Shen J, Conti CJ, Lanari C & Molinolo A 2002 p21, p27 and p53 in estrogen and antiprogestin-induced tumor regression of experimental mouse mammary ductal carcinomas. *Carcinogenesis* **23** 749–758. (<https://doi.org/10.1093/carcin/23.5.749>)
- Wargon V, Helguero LA, Bolado J, Rojas P, Novaro V, Molinolo A & Lanari C 2009 Reversal of antiprogestin resistance and progesterone receptor isoform ratio in acquired resistant mammary carcinomas. *Breast Cancer Research and Treatment* **116** 449–460. (<https://doi.org/10.1007/s10549-008-0150-y>)
- Wargon V, Fernandez SV, Goin M, Giulianelli S, Russo J & Lanari C 2011 Hypermethylation of the progesterone receptor A in constitutive antiprogestin-resistant mouse mammary carcinomas. *Breast Cancer Research and Treatment* **126** 319–332. (<https://doi.org/10.1007/s10549-010-0908-x>)
- Wargon V, Riggio M, Giulianelli S, Sequeira GR, Rojas P, May M, Polo ML, Gorostiaga MA, Jacobsen B, Molinolo A, et al. 2015 Progesterone and antiprogestin responsiveness in breast cancer is driven by the PRA/PRB ratio via AIB1 or SMRT recruitment to the CCND1 and MYC promoters. *International Journal of Cancer* **136** 2680–2692. (<https://doi.org/10.1002/ijc.29304>)