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Mifepristone for treatment of uterine leiomyoma. A prospective randomized placebo controlled trial

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BACKGROUND: Uterine leiomyomas are widely prevalent and frequently cause menorrhagia. The major therapeutic option today is hysterectomy. Medical options are of highest interest.

METHODS: A total of 30 women with uterine leiomyomas scheduled for surgical intervention were randomized to receive either 50 mg mifepristone or placebo every other day during 3 months prior to surgery. Uterine blood flow and leiomyoma volume were evaluated once a month until surgery. Endometrial biopsies were obtained prior to and at end of treatment. Relevant biochemistry, symptoms and bleeding were recorded. Primary outcome was reduction in uterine leiomyoma size.

RESULTS: There was a significant percentual decrease ($P = 0.021$) in the total leiomyoma volume in the mifepristone-treated group, −28 (−48, −8) % (mean ± 0, 95 confidence interval), compared with the control group values 6 (−13, 25) %. Mifepristone treatment significantly reduced the bleeding days ($P = 0.001$) and increased serum haemoglobin values ($P = 0.046$). Serum cortisol levels remained unchanged, while a mild increase in serum androgens was noted. Endometrial biopsies showed no premalignant changes or changes in mitotic indices.

CONCLUSION: Mifepristone may offer an effective treatment option for women with uterine leiomyoma and the associated pronounced uterovaginal bleeding.
Clinical Trials identifier: www.clinicaltrials.gov: NCT00579475.

Key words: mifepristone / leiomyoma / bleeding / endometrial morphology

Introduction

Uterine leiomyoma is one of the most frequent causes of menorrhagia leading to significant compromise in quality of life, in women during their reproductive age. Excessive vaginal bleeding, pressure related discomforts and infertility with pregnancy complications lead to surgical procedures in these patients (Wallach and Vlahos, 2004). The incidence of hysterectomy due to leiomyoma is 2/1000 women/year or 200 000/year which represents one third of all hysterectomies in the USA (Wilcox *et al.*, 1994). Thus the individual discomfort and the healthcare cost for society due to uterine leiomyoma is of significant importance. Among Afro-American women, the prevalence is even higher as well as the incidence of surgical procedures. Examination of hysterectomy specimens showed that 89% of Afro-Americans and 59% of the white women had uterine fibroids (Kjerulff *et al.*, 1996). Various minimal invasive methods such as catheter-guided embolization of the leiomyoma feeding vessels or abdominal surgery with laparoscopic methods are still beyond reach for most patients because highly developed operative skill and advanced technical requirements are necessary. Long-term use of gonadotrophin releasing hormone agonist (GnRHa) treatment is problematic because of significant side effects such as flushes and bone resorption following hypo-estrogenic effect (Friedman *et al.*, 1988; Johansen *et al.*, 1988). Providing estrogen as add-back therapy neutralizes the favourable effect on fibroid size reduction (Friedman *et al.*, 1993).

Exposure to estrogen and progesterone promotes the growth of uterine leiomyomas (Flake *et al.*, 2003). Therefore treatment with the antiprogesterone drug mifepristone, as well as other progesterone receptor modulators (PRMs), has been evaluated. A daily low-dose treatment with mifepristone (5 mg/day for 26 weeks), improved the

score for fibroid-specific quality of life (Fiscella *et al*., 2006). More recently developed PRMs have been shown to reduce leiomyoma size and symptoms in a dose-dependent fashion (Chwalisz *et al*., 2007; Levens *et al*., 2008). None of these compounds is currently available for clinical use.

Mifepristone is a well-studied antiprogestin, which has been in use for over two decades for various clinical indications (Gemzell-Danielsson *et al*., 1993; Gemzell-Danielsson and Marions, 2004; Fiala *et al*., 2007; Lalitkumar *et al*., 2007). The effect of mifepristone on follicular development, ovulation, endometrial development and function is dependent on dose and timing of exposure. Treatment immediately after ovulation inhibits endometrial development and receptivity while mid luteal treatment induces uterine bleeding in a dose-dependent manner (Gemzell-Danielsson *et al*., 1994). Low doses of 2–5 mg per day resulted in anovulation and inhibition of menstruation in over 90% of menstrual cycles, with decreased endometrial proliferation in healthy fertile women (Baird *et al*., 2003a, b; Cameron *et al*., 1996). Reduced bleeding has been reported during mifepristone treatment with doses between 5 and 50 mg daily (Murphy *et al*., 1993, 1995; Steinauer *et al*., 2004; Lakha *et al*., 2007).

The aim of the present study was to evaluate the effect on leiomyoma volume, endometrium and bleeding during 3 months of mifepristone treatment, by performing a prospective randomized, placebo-controlled, double-blinded 'proof of concept' study including side effect monitoring.

Patients and Methods

This prospective, randomized, placebo-controlled study was conducted at the Karolinska University Hospital, Stockholm, Sweden after obtaining approvals from the Ethics committee at Karolinska Institutet. Healthy, non-pregnant women, referred for evaluation to the outpatient clinic due to leiomyoma related problems indicating surgical intervention, were considered eligible for this study. All women gave their written informed consent prior to inclusion. Reasons for surgery included menorrhagia, pressure related symptoms from bladder or bowel or a considered risk for pregnancy complications. None of the women had used any steroid hormonal therapy for a minimum of 3 months prior to recruitment. The exclusion criteria were any history of breast cancer or other malignancy; bleeding not possible to control with tranexamic acid and iron medication, implicating a need for urgent surgical treatment; abnormal mammogram and breast biopsy at the baseline investigation; adnexal abnormality, or suspicion of leiomyosarcoma upon transvaginal ultrasound examination; abnormal FSH and LH-levels or any other hormonal dysfunction of clinical significance; laboratory findings that would give suspicion of blood, liver or renal dysfunction; abnormal PAP smear at screening; or any contraindication to the use of mifepristone. The women were instructed to use barrier methods of contraception unless sterilized or having a vasectomised partner. Blood sampling for hormonal status was performed at baseline and after 3 months. Pelvic vaginal ultrasound was conducted on average 6 days prior to the commencement of treatment and was repeated every fourth week during the treatment period. The uterine and leiomyoma volumes were determined by ultrasound examination and Doppler investigation of pulsatile index (PI) and peak flow in the uterine artery and the leiomyoma feeding vessels was performed. Assessments of haematological, renal and liver laboratory data were made every fourth week during the study duration. Breast biopsies were obtained at study entry and at the end of the study as reported elsewhere (Engman *et al*., 2008). Endometrial biopsies were obtained at baseline, and after the treatment period during surgery.

Treatment schedule

Women fulfilling the inclusion criteria were randomized into two treatment groups using a computer-generated, randomized, double-blinded selection procedure which was done by the Karolinska University Hospital Pharmacy. Medication was packed and coded by the Pharmacy according to the randomization list. Patients and staff were blinded to treatment groups. The patients received either mifepristone 50 mg (one quarter of 200 mg, Mifegyne®, Exelgyn, Paris, France) as active substance or visually identical B-vitamin tablets (one quarter of TrioBe® Recip, Stockholm, Sweden) as placebo every other day starting on the first day of the menstrual cycle. The duration of treatment was 3 months, which ended on the day before surgery. The women returned all used and unused study pill packages at the end of treatment visit.

Ultrasonographic Doppler examination

The vaginal ultrasound and Doppler investigation was conducted using a vaginal probe for colour Doppler imaging and spectral analysis using Voluson730 Expert (General Electric, Zipt, Austria) equipment. All examinations were made or supervised by the same operator. A defined protocol for the ultrasound investigation was used for each patient before the start of medication and carried out every fourth week until the surgery. The PI and peak-flow (cm/s) were measured along with endometrial thickness, localization and number of leiomyomas. The largest myoma diameter for each myoma was used for size ranking 1–5. The three largest diameters (A, B and C) were measured in two planes in approximately perpendicular *x*, *y* and *z*-axis directions in up to five leiomyomas. The shapes of myomas are spherical or ellipsoid depending on the location relative to the cavity or the myometrial wall the volume was calculated using the formula for an ellipsoid, $0.523 \times A \times B \times C$. Impedance of blood flow was expressed as the PI, using a flow velocity waveform according to the formula: PI = (S−D)/Time-average maximal flow (TAMAX) velocity during the cardiac cycle. The peak flow in systole (S), diastole (D) and TAMAX was expressed as centimetre/second. The hemodynamic parameters PI and peak-flow were measured in the uterine arteries, as well as in the peripheral and central leiomyoma vessels, at a probe angle reflecting the highest pulsatility. Occasionally, an abdominal probe was used, when the leiomyoma reached beyond the pelvic borders.

For control of intra-observer agreement the volume of myomas at surgery were calculated from the mid-sectional diameter, and correlated to the volume of myomas assessed by ultrasonography prior to surgery.

Symptom registration

All women were asked to keep daily records of bleeding and symptoms such as pain or pressure during the study period. Any side effects were noted, as well as any medication used at the baseline or during the study. The participants were asked to report leiomyoma-related and general symptoms once weekly graded on a five-point Likert scale (0 = no symptoms, 1 = light, 2 = moderate, 3 = severe and 4 = very severe). Symptoms were reflecting two categories; local symptoms such as pelvic pain or pressure, bladder pressure, micturition problems, lower back pain, proctodynia and dyspareunia, and general hormone-related symptoms such as flushes, headache, nausea, vomiting, diarrhoea, mood fluctuation, libido, weakness or fatigue. The scores (0–4) were registered weekly and summarized for every 4 week period (range 0–16) and compared within and between the treatment groups at 4, 8 and 12 weeks of treatment. The scores for local and general symptom category were also summarized and analyzed by category.

In addition, values reflecting first week from baseline were compared with the last treatment week.

Laboratory and safety data

Routine blood parameters such as haemoglobin and white blood cell count as well as liver transferase enzymes were monitored after every 28 day period during the treatment period. Estradiol, progesterone, testosterone, sexual hormone binding globulin, androstenedione, dehydroepiandrostendione, FSH, LH, prolactin, thyroid hormones and 24 h urinary cortisol excretion were assessed at baseline and at the end of study. An endometrial biopsy was obtained using a Randall curette (Stille, Sweden) before start of medication and after the treatment period during surgery. Mammography was performed at start of the study and fine-needle breast biopsies were obtained at baseline and closely before the scheduled surgical event.

Endometrial morphology, proliferation and steroid receptor expression

Endometrial biopsies obtained from the surgical specimen at baseline and after 3 months exposure to mifepristone or placebos were evaluated by a blinded expert investigator. In 8 of 12 samples in the active group and 11 of 14 samples in the control group it was possible to evaluate morphologic structure and mitotic activity index. In some patients difficulties in obtaining a good biopsy was noted, possibly due to the location of leiomyomas, that occasionally severely distorted the cervical canal.

Morphological analysis

Endometrial sections stained with haematoxylin–eosin were assessed by a pathologist experienced in evaluating selective progesterone receptor modulator-associated endometrial changes, blinded to the treatment groups. The sections were assessed for overall histological patterns, and for glandular and stromal mitotic index, cystic glandular dilatation and for abnormality of blood vessels. Biopsies were assessed as non-physiological if there was abnormality of glandular architecture including cystic dilatation, asynchrony of glandular and stromal secretory differentiation, prominent ciliated or eosinophilic metaplastic change in glandular epithelium, or abnormal stromal vessels. Glandular and stromal mitotic indices were assessed in a semi-quantitative way and scored from 0 to 3 (0 = none, 1 = infrequent but present, 2 = present in moderate numbers, 3 = frequent).

Immunohistochemistry

Expression of steroid hormone receptors and Ki-67 as proliferation marker were studied by immunohistochemistry in the luminal, glandular and stromal compartments of the endometrium. The tissue sections were deparaffinized and quenched with hydrogen peroxide to inactivate the tissue peroxidase activity. Following rinse in tris-buffer saline (TBS), sections were incubated with the primary antibody of interest over night at 4°C. After incubating with biotinylated secondary antibody and rinsing in TBS, the sections were treated with avidin–biotin–peroxidase complex (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, USA) for 30 min followed by 3,3′-diaminobenzidine tetrahydrochloride (Peroxidase Substrate Kit, Vector Laboratories, Inc., Burlingame, USA). A negative control excluding the primary antibody was included in every batch. After light counterstaining with haematoxylin, the sections were evaluated by two independent blinded investigators using an immunoreactive scoring (IRS) system, a semi-quantitative subjective scoring system based on both percentual distribution (PP) and staining intensity (SI) as follows. IRS = SI * PP, of which SI is the visually graded SI (graded as 0: no staining; 1: weak staining; 2: moderate staining and 3: strong staining) and PP is the percentage of cells stained positive. The PP was estimated by counting approximately 200 cells and was scored as 0: no staining; 1: <10% staining; 2: 11–50% staining; 3: 51–80% staining; and 4: >81% staining (Mylonas *et al.*, 2004).

As Ki-67 immune staining was very heterogeneous with same intensity and relatively few cells were positive, the scoring template reference was modified as follows: 0: no cell stained; 1: <5% of cells; 2: 5–10% of cells; 3: 10–20% of cells and 4: >20% of cells positively stained.

Statistical analysis and sample size calculation

Sample size was calculated with the main outcome parameter as reduction in uterine leiomyoma size. Assuming a standard deviation of 10% in the percentage volume change, 18 subjects per group would be required to detect a difference of at least 10% in the percentage volume change between the treated and the placebo groups with 90% power, using a one-sided 5% level test. Allowing for a 10% drop out, 20 subjects per group, or a total of 40 women were planned to be recruited into the trial.

Significance within and between the groups was analyzed using Wilcoxon signed-rank test and Mann–Whitney *U*-test, respectively. Correlations were assessed by Spearman's rank correlation test. A *P*-value of <0.05 was considered as significant.

Results

Demographic data

Women were enrolled from November 2004 to June 2007. A total of 72 women were eligible for the study. Among them 40 women declined from participating as they wished urgent relief from the leiomyoma-related problem. Due to reorganization of the clinic recruitment had to stop when 30 women had been randomized. After completing the study, on breaking the code, it was found that 14 patients received mifepristone and 16 placebo treatment. During the study two women in the placebo group were excluded after randomization due to uncontrollable bleeding in one case and elevated serum FSH in the other. Fourteen patients in each group remained throughout the study duration (Fig. 1). The mean number of treatment days ($\pm$SEM) was 85 $\pm$ 1 in the mifepristone group and 83 $\pm$ 2 in the control group.

The mean age ($\pm$SD) was 40.9 $\pm$ 7.6 and 40. 8 $\pm$ 4.7, respectively, in the control group and mifepristone group. Parity median (range) was 0 (0–1.5) for the control group and 0.5 (0–2) for the mifepristone group while BMI (median, interquartile) was 22.3 (21.3–24.1) in the control group and 25.9 (24.8–29.8) in the mifepristone-treated group. There were no significant differences in age, parity, BMI or ultrasonographic endometrial double layer thickness between the groups at baseline (Table I).

Effects on leiomyoma volume and uterine blood flow

The median (interquartile range) volume (ml) of the dominant leiomyoma at baseline was 97 (42–192) in the control group and 137 (111–163) in the mifepristone-treated group. This difference was not significant. The volume of the dominant leiomyoma exceeded 375 ml in only one patient per treatment group. The median (range) number of leiomyomas at baseline was 2.0 (1–4) in the control group and 1.5 (1–4) in the mifepristone group. The volume (ml) of

the dominant leiomyoma changed from baseline until the end of study in the control group median (interquartile range) from 97 (42–192) to 85 (68–160) and in the mifepristone group from 137 (111–163) to 96 (42–129) and was significantly reduced ($P = 0.028$) within the mifepristone group. The percentual delta volume of the dominating myoma and total bulk of myoma was normally distributed with a skewness less than ± 1 and therefore permits to be analyzed by means within a 0.95 confidence intervals. The percentual delta volume of the dominating myoma was significantly reduced ($P = 0.014$) with mifepristone treatment, that gave a reduction of mean within 0.95 confidence interval -27 (-47 to -8) % and in the control group the mean within 95% CI percentual delta volume of the dominant myoma was $+8$ (-10 to $+26$) %.

The total leiomyoma bulk (ml) was reduced in the control group median (interquartile range) from 134 (53–196) to 118 (80–160). Within the mifepristone group a significant reduction ($P = 0.023$) in the volume of the total leiomyoma bulk from baseline 161 (111–209) to the end of study 106 (52–205) was found. There was a significant decrease ($P = 0.021$) in the percentual total leiomyoma delta volume from baseline to the end of study in the mifepristone-treated group (mean within 95% CI) -28 (-48 to -8) %, compared with the control group values 6 (-13 to $+25$) % (Table II).

The mean total uterine volume in this study was not significantly reduced by mifepristone treatment.

We found no significant differences in the impedance (PI) or peak-flow between or within the treatment groups at baseline or end-point, at any of the measurement points.

For control of intra-observer agreement the volume of myomas at surgery were calculated from the mid sectional diameter, and correlated to the volume of myomas assessed by ultrasonography prior to surgery. The correlation found to be significant ($P < 0.001$, $r = 0.8$).

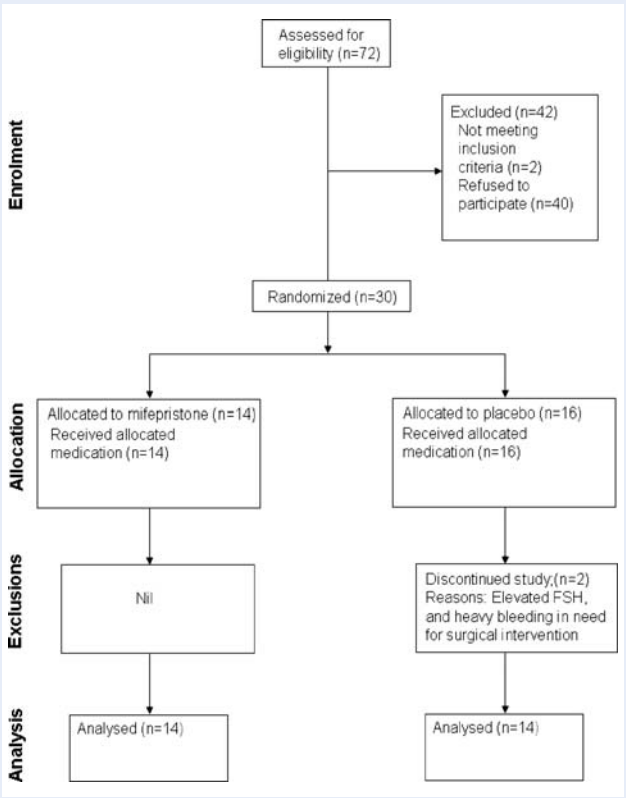

Figure 1 Study flow chart: patient enrolment details in placebo and mifepristone treated groups.

Effects on uterine bleeding and patient reported symptoms

There were no significant differences between the placebo and mifepristone-treated groups concerning bleeding pattern at baseline reflecting the three different categories of indication for surgery. The

Table I Baseline characteristics of randomized subjects (expressed as mean ± SD) if skewness less than ±1, if not as median within IQR = interquartile range

Parameter	Control ($n = 16$)	Mifepristone ($n = 14$)	Significance *P*-value
Age	40.9 ± 7.6	40.8 ± 4.7	n.s
Parity (median IQR)	0 (0–1.5)	0.5 (0–2)	n.s
BMI(median IQR)	22.3 (21.3–24.1)	25.9 (24.8–29.8)	n.s
Endometrial double layer thickness (mm)	7.2 ± 5.1	6.5 ± 4.9	n.s
Progesterone nmol/l (Post-menopausal <3.0)	22.1 ± 14.9	20.5 ± 13.3	n.s
Estradiol pmol/l (Luteal phase 300–1000)	362 ± 215	421 ± 188	n.s
Testosterone nmol/l (Ref. <2.7)	1.13 ± 0.76	1.43 ± 0.61	n.s
Free testosterone (f) nmol/l	0.014 ± 0.008	0.020 ± 0.007	0.04
SHBG nmol/l (Ref. 35–150)	81.6 ± 37.2	62.2 ± 33.9	n.s
Androstenedione nmol/l (Ref. 1.6–12)	5.03 ± 2.29	5.84 ± 2.59	n.s
FSH U/l (Ref. lutealphase 1–12)	6.23 ± 9.09	4.59 ± 3.16	n.s
LH U/l (Ref. lutealphase: 1–20)	4.78 ± 5.46	7.15 ± 9.19	n.s
DHEAS μmol/l (Ref. 1.6–9.5)	5.04 ± 2.64	5.89 ± 1.96	n.s
Pt-U cortisol nmol/24 h (Ref. 70–500)	173 ± 55	189 ± 89	n.s

SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone.

Table II Effect of mifepristone on the dominant myoma and total myoma volume expressed as median within IQR

Volume (ml)	Control			Mifepristone		
	Baseline ($n = 16$)	End of study ($n = 15$)	Sig. P	Baseline ($n = 14$)	End of study ($n = 12$)	Sig. P
Dominant myoma IQR	97 (42, 192)	85 (68, 160)	n.s.	137 (111, 163)	96 (42, 129)	0.03[a]
Delta volume (ml) IQR (ml)		3 (−37, 19)	n.s.		−29 (−60, −7)	n.s.
% Change (mean) Confidence interval		8 (−10, 26)	0.01[b]		−27 (−47, −8)	0.01[b]
Total myoma volume(ml) IQR	134 (53, 196)	118 (80, 160)	n.s.	161 (111, 209)	106 (52, 205)	0.02[a]
Delta total myoma volume (ml)		−8 (−37, 20)	n.s.		−36 (−62, −5)	n.s.
IQR (ml)		0.11			0.11	
% Change (mean) Confidence interval		6 (−13, 25)	0.02[b]		−28 (−48, −8)	0.02[b]

Deltavalues between baseline and end of study expressed as (ml). The percentual change (%) was distributed with skewness less than ± 1 permitting means within 95% confidence interval.
[a]*P*-value within group between baseline and end of study.
[b]*P*-value between groups at the end of study.

Table III Indications for surgery and bleeding pattern (median, range) at baseline

Indication for surgery	Control ($n = 16$)			Mifepristone ($n = 14$)		
	n	Median bleed days (Range)	Median cycle length (Range)	n	Median bleed days (Range)	Median cycle length (Range)
Heavy bleeding	9	8 (5–9)	28 (26–30)	5	6.5 (5–8)	28 (28–29)
Bulk dependent symptoms	5	4 (3–6)	28 (26–30)	5	6 (3–7)	28 (24–30)
Dysfertility	2	4 (3–5)	30 (28–32)	4	4.5 (3–8)	28 (24–35)

Data from two patients excluded from control group due to reasons explained under demographic data were incorporated into baseline analysis as they were recruited with the intention to treat.

patients were distributed in a similar manner to the indication categories without any significant inter category difference (Table III).

Through the first 4 weeks of treatment the placebo group had 7.5 (2–19) median bleeding days and the mifepristone-treated group 7 (4–10). A highly significant ($P = 0.001$) reduction in bleeding days occurred during week 5–8 within the mifepristone group compared with baseline median (range) value 0 (0–2) or compared with the placebo group median 7.5 (3–26) ($P = 0.001$). Only two women in the mifepristone group reported any bleeding for 1 and 2 days during week 5–8. Only one of these patients reported spotting at one occasion during week 9–12 of treatment while the placebo treated group had 3 (0–27) median (range) days of bleeding compared with the mifepristone-treated group ($P < 0.001$). Thus, the number of mifepristone treated patients completely free of vaginal bleeding was 86% during the second treatment month and close to 100% during the third month of treatment. There was no significant change of the bleeding pattern within the control group (Fig. 2).

The symptom scores reported for flushes showed significant changes ($P = 0.02$) within the mifepristone-treated group where scores for flushes increased from median (range) 0.5 (0–7) to 3.5 (0–16) on a scale from 0 to 16 during treatment. Between the groups a significant difference in flushes was noted ($P = 0.012$) at the end of the study. No other intra- or inter-group differences in symptoms were detected (Table IV).

Effects on haemoglobin, safety data and hormonal levels

At baseline there was no significant difference in blood haemoglobin values (mean ± SD) between the groups (124 ± 16 versus 121 ± 17) (Table I). Haemoglobin counts improved significantly ($P = 0.003$) from 121 ± 5 g/l to 133 ± 3 ml (mean ± SE) within the mifepristone group from baseline to the end of study. Within the control group haemoglobin was unchanged from 124 ± 16 to 122 ± 17. Between the groups at the end of study the difference between the groups was significant ($P = 0.046$) (Table V).

No significant changes were observed in the liver transferase enzyme profile, except for one patient in the mifepristone-treated group that had an isolated slight baseline elevation of alanine aminotransferase (1.190), she was included and carefully monitored (at 4, 8 and 12 weeks) and was considered unaffected by treatment. About 24 h urine cortisol level was not affected during 3 months treatment with mifepristone. No differences between the groups in hormonal levels were noted at baseline, except for slightly higher levels of

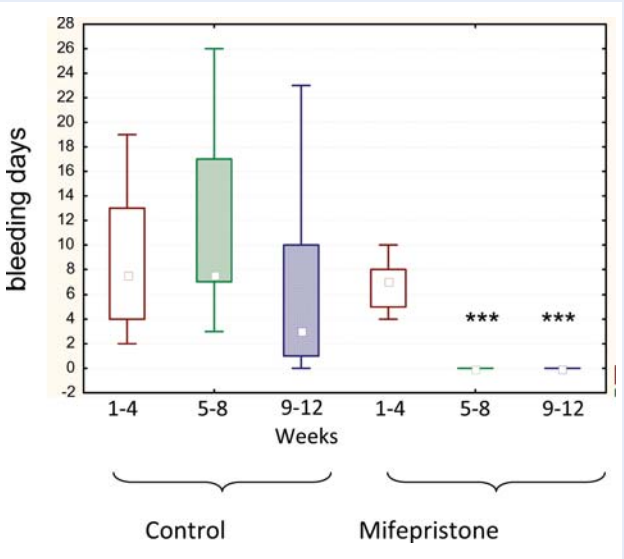

Figure 2 Vaginal bleeding profile of women exposed to mifepristone or placebo.

There was highly significant reduction in bleeding days in the mifepristone group during week 5–8*** ($P = 0.001$) and week 9–12*** ($P = 0.001$) of treatment. There was no significant difference between the groups during the first 4 weeks of treatment (median, box; 25–75%, whiskers; non-outlier range).

free testosterone in the mifepristone group (Table I). Even though the levels of free serum testosterone and androstenedione were within the normal reference limit ranges, there was an increase in free testosterone (mean ± SD) within the mifepristone group from 0.020 ± 0.007 to 0.026 ± 0.009 ($P = 0.038$) and for the control group 0.014 ± 0.008 to 0.014 ± 0.006. The change was significant ($P = 0.002$) between groups. Also the androstenedione level was elevated within the mifepristone group ($P = 0.008$) but not in the control group. Between the treatment groups a significant difference was seen ($P = 0.002$) at the end of study. The serum estradiol level decreased significantly from the base line mean (± SD) value of 421 ± 188 to 196 ± 30 (pmol/l) by the end of treatment within the mifepristone group ($P = 0.004$). In the control group mean (± SD) estradiol was unchanged, 362 ± 215 at baseline and 386 ± 420 at the end of study. The level of progesterone decreased significantly in the mifepristone group from 20.4 ± 13.6 to 2.2 ± 0.6 nmol/l ($P = 0.005$) and also in the control group from 22.1 ± 14.9 at baseline to 8.5 ± 9.6 at the end of study ($P = 0.013$). Progesterone but not estradiol or free testosterone levels significantly correlated ($P = 0.005$) with the scores for flushes. Detailed hormonal data has been previously reported (Engman *et al.*, 2008).

Effects on the endometrium

Endometrial biopsies were collected at baseline and at the time of surgery. It was possible to evaluate biopsies from 8 of 12 in the mifepristone group and 11 of 14 in the control group. None of the biopsies showed any evidence of hyperplasia or malignancy. Of the eight mifepristone cases, seven showed non-physiological appearances compared with 4 of 11 assessable placebo cases (statistically not significant). Cystic glandular dilatation was significantly more frequent in mifepristone-treated women, present in 5 of 8 cases compared with in 1 of 11 placebo cases ($P = 0.041$). No significant differences were observed between treatment groups in mitotic index of glands or stroma, or in frequency of present abnormal vessels (Fig. 3).

At surgery endometrial biopsies were taken for immune histochemistry analysis. The staining of cell proliferation marker Ki-67, estrogen receptor alpha and beta (ER-α and ER-β) progesterone receptor B (PR-B) and progesterone receptor A+B (PR (A+B)) and androgen receptor (AR) were localized in the nucleus. At the end of study mifepristone significantly down-regulated the expression of Ki-67 in the stromal compartment compared with placebo treatment ($P = 0.026$). In the glandular epithelial cells mifepristone increased the expression of PR (A+B), PR-B, AR and ER-α ($P = 0.039$, 0.017, 0.04 and 0.012, respectively) whereas PR (A+B) was also increased ($P = 0.03$) in luminal epithelial cells compared with the placebo group. No differences between the groups were observed in the expression of ER-α and -β, PR (A+B), or PR-B in the stromal compartment (Fig. 3).

Discussion

The results of the present study demonstrate that 3 months treatment with mifepristone 50 mg on alternate days results in a significant reduction in leiomyoma size, reduced uterovaginal bleeding and increased blood haemoglobin. Excessive vaginal bleeding is often the sole symptom reported by women with leiomyomas (Bukulmez and Doody, 2006). The severity of the bleeding may be sufficient to cause iron-deficiency anaemia. Heavy bleeding also confines women with social isolation psychological discomfort and loss of productive time (Stovall, 2001) In a random sample of 878 women aged 35–49 years, 28% of the women who reported bleeding disorder had no detectable leiomyoma, 64% had leiomyomas of whom 46% of the women with leiomyoma reported gushing of blood during their menstrual cycle. In these women the size of the leiomyomas had a significant contribution to the severity and length of bleeding (Wegienka *et al.*, 2003). The present study treatment with 50 mg of mifepristone on alternative days shows that this treatment drastically and rapidly controls uterine bleeding, all but one women become amenorrhoic in the third 28-day treatment period and only two women reported 1–2 single days of bleeding each during the second 28-day treatment period. Thus, the most important and useful effect of mifepristone appears to be the control of uterovaginal bleeding leading to the improvement of blood haemoglobin levels. In recent placebo-controlled studies with other PRMs for uterine leiomyoma treatment both Asoprisnil and Ulipristal showed a dose-dependent degree of amenorrhea which was less pronounced than with the currently studied regimen of mifepristone (Chwalisz *et al.*, 2007; Levens *et al.*, 2008). In this study we used 50 mg of mifepristone every other day. Earlier studies with mifepristone have also shown a dose-dependent induction of amenorrhea. Treatment with 5–10 mg of mifepristone for 1 year led to amenorrhea in 40–70% of patients, while treatment with 100 mg of mifepristone led to 100% amenorrhea (Kettel *et al.*, 1994; Eisinger *et al.*, 2003). A potential disadvantage with higher doses of mifepristone compared with the newly developed PRMs is the antiglucocorticoid effect. Thus, taking these factors, the half-life (about 24 h) and the available dosage in the pharmacy into account,

Table IV The number of women (*n*) that changed their Likert score from baseline compared with the last treatment week

Symptoms	Increased side effects (Total no. of patients = 14)		Decreased side effects (Total no. of patients = 14)	
	Control (*n*)	Mifepristone (*n*)	Control (*n*)	Mifepristone (*n*)
Pelvic pain	0	3	5	2
Bladder pressure	3	1	1	4
Micturition problem	0	1	1	1
Lower back pain	1	1	2	3
Proctodynia	0	1	4	2
Coital pain	0	2	1	0
Flushes	0	6	4	1
Headache	2	3	5	4
Nausea	1	2	3	3
Vomiting	0	0	2	1
Diarrhea	0	1	0	3
Change of mood	1	7	3	3
Lowered libido	1	4	3	1
Weakness	2	4	3	3
Fatigue	3	4	5	5

The only significant change ($P = 0.02$) was the score for flushes in the mifepristone group.

Table V Blood and parenchymatous organ safety parameters before and after 3 months treatment (mean ± SD)

Parameter (Reference values)	Control		Mifepristone		Significance
	Baseline	End of study	Baseline	End of study	*P* (b)
Haemoglobin (117–153 g/l)	124 ± 16	122 ± 17	121 ± 17	133 ± 11	$P = 0.046$[a], $P = 0.003$[b]
ASAT(Aspartate aminotransferase) (<0.61 μkat/l)	0.38 ± .20	0.30 ± .10	0.29 ± 0.17	0.30 ± .11	n.s
ALAT (Alanine aminotransferase) (<0.76 μkat/l)	0.29 ± .11	0.33 ± .14	0.36 ± 0.28	0.41 ± .29	n.s
ALP (Alkaline phosphatase) (<1.9 μkat/l)	1.20 ± .65	0.89 ± .28	1.31 ± 0.81	0.96 ± .41	n.s
Creatinin (<90 μmol/l)	60 ± 12	59 ± 7	60 ± 11	61 ± 9	n.s
Pt(U)-cortisol (36–250 nmol/24 h)	173 ± 55	149 ± 75	189 ± 89	164 ± 80	n.s

[a]Significant change ($P = 0.003$) within mifepristone group from baseline to end of study.
[b]Significant difference between treatment groups at the end of study.

we decided to administer 50 mg of mifepristone every other day as one of the primary aims of the study was to achieve relief from leiomyoma related heavy bleeding. With this regimen 24 h urine excretion of cortisol was unaffected, as were the other safety laboratory parameters studied. This is the first report showing that treatment with mifepristone can increase haemoglobin level. If used before surgery mifepristone could be administered to reduce the bleeding and improve the haemoglobin level, thus potentially avoiding the need for blood transfusion. However, ulipristal (CDB-2914) treatment did not result in improved haemoglobin levels, despite a reduction in bleeding (Levens *et al.*, 2008).

While a higher dose of mifepristone seems to be needed for the induction of amenorrhea in patients with leiomyomas, even low daily doses have been shown to reduce leiomyoma volume. A daily 5 mg of mifepristone dose resulted in amenorrhea in only 41% of women but gave a 47% reduction in uterine size (Fiscella *et al.*, 2006).

In the present study the volume of the largest leiomyoma and the sum of all coexisting leiomyoma volumes were significantly reduced. The leiomyoma volume was measured using ultrasound. We agree with earlier reports that ultrasound volumetric measurement has shown limitations in terms of accurately evaluating larger (>375 ml) and multiple (>4) leiomyomas (Dueholm *et al.*, 2002). Magnetic resonance imaging (MRI) may be a better tool for reproducibility and mapping, especially multiple large leiomyomas and for following their growth over time, with presumably a better possibility to 'tag' the individual tumours measured (Levens *et al.*, 2008). However, to study hemodynamic parameters, the use of ultrasound Doppler is superior as no validated method for measuring blood flow and impedance is currently available with MRI.

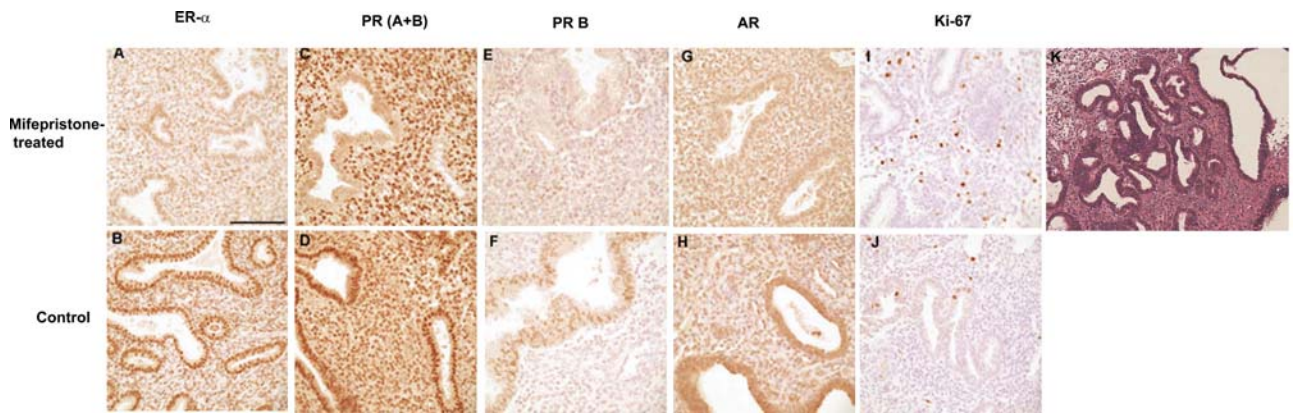

Figure 3 Immunohistochemical analysis of endometrial ER-α (**A, B**), PR A + B (**C, D**), PR B (**E, F**), AR (**G, H**) and Ki67 (**I, J**) in placebo (A, C, E, G, I) and mifepristone (B, D, F, H, J) exposed patients.

A significant increase in the immunostaining for steroid receptors was observed in glandular epithelial cells of mifepristone-treated group while the expression of Ki-67 was decreased. Endometrial biopsy from mifepristone-treated patient (**K**), showing irregularity of gland architecture with cystic dilatation and others a tubular morphology with focal crowding. The glandular epithelium is of rather inactive appearance, with minimal nuclear stratification. The stroma is mainly compact but non-decidualized, with thin-walled vessels present. Bar = 100 μ.

Another limitation of this study is that the total uterine volume cannot be considered ellipsoid in shape when there are multiple or larger leiomyomas especially when the localization is predominantly subserous representing a rather different spatial shape between individuals and possibly between measurements. In our observations we did not notice any significant reduction of the total uterine volume as a result of treatment. Reduction of total uterine volume has been noticed by other investigators with this drug (Fiscella *et al*., 2006).

The hemodynamic changes studied with Doppler measurements of the uterine and leiomyoma feeding vessels showed no significant differences between the treatment groups or any correlations with hormonal change of status and leiomyoma volume changes, although there was a tendency towards increased impedance and reduction of flow at all measured points. The individual variation was pronounced reflecting the fact that small variations in values created important differences in percent changes and pronounced variations because of the low amplitude of graphical presentation of blood flow velocity curves in the small vessels supporting the leiomyomas. Gn-RH agonist treatment has previously been shown to give a significant reduction in flow and increase of impedance over time, starting in the leiomyoma vessels and followed in the uterine vessels, an effect strongly correlated with decreasing estradiol values during 12 weeks treatment (Aleem and Predanic, 1995). In our study we did not find any significant correlations of estradiol, progesterone, testosterone or androstenedione with the impedance or peak flow leiomyoma volume or other parameters such as bleeding days and haemoglobin changes. The mechanism for reduced bleeding and leiomyoma volume seems to be explained in another manner than hemodynamically, and is more likely due to the structural, functional and micro vascular effects of mifepristone on the endometrium and possibly leiomyoma tissue.

It has been observed in earlier studies that, during continuous daily treatment with PRMs, specific endometrial changes occur such as a mixed degree of proliferation and apoptotic changes in specifically structured dilated endometrial glands (Mutter *et al*., 2008). In agreement with previous studies some degree of specific cystic glandular dilatation was also observed in this study. Earlier studies have also reported that the changes seen in the endometrium are reversible on discontinuation of treatment (Newfield *et al*., 2001). We observed a down-regulation in the expression of Ki-67 which is concurs with earlier studies of long-term mifepristone treatment (Baird *et al*., 2003a, b). The slight but significant increased levels of testosterone and androstenedione may be relevant to the observed changes in endometrial morphology and the induction of amenorrhea (Brenner *et al*., 2003). Earlier studies have shown that the expression of AR was increased in the epithelial compartment with mifepristone treatment (Baird *et al*., 2003a, b; Brenner *et al*., 2003) as seen in this study. Early studies showed that mifepristone binds to AR (Moguilewsky and Philibert, 1985). The mechanism for increasing the androgen expression could be a discrete activation of the hypofys-adrenal axis without obvious effect on the cortisol 24 h urinary excretion or by a block of the ovarian aromatase. Endometrial mifepristone blockage of aromatase was reported (Tseng *et al*., 1986) and could possibly have a role in the AR associated antiproliferative effect in endometrium by mifepristone. The steroid hormone concentrations over time during exposure are reported in a previously published article concerning effects in breast tissue from the same patients as discussed here (Engman *et al*., 2008).

The leiomyoma specific symptoms surprisingly did not differ between the groups possibly due to low sensitivity of the questionnaire used in the study. Using a validated Quality of Life questionnaire might have given more detailed information. In a previous study a significant effect was shown with 5 mg daily of mifepristone on the quality of life for women with uterine leiomyomas (Fiscella *et al*., 2006).

The current proof-of-concept study supports the use of mifepristone (50 mg administered every other day) as an effective, presurgical management of leiomyoma-related uterovaginal bleeding. This treatment strategy induces amenorrhea and increases haemoglobin

levels, along with reducing leiomyoma volume. The reduction in the size of leiomyomas was observed without concurrent reduction in uterine blood perfusion. Further studies are needed to understand the regulatory pathways and mechanisms involved in the size reduction of leiomyoma and the mechanism of action of mifepristone. This short-term treatment was well-tolerated with no adverse effects on cortisol levels and only a mild degree of hot flushes reported in some women. Although further, larger, long-term studies are needed to add safety information, mainly concerning endometrial and breast proliferation, the present proof-of-concept study provides valuable evidence for the possibility of using the well-studied anti-progestin mifepristone as a medical treatment option of leiomyoma as well as for the treatment of menorrhagia.

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