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AUTHORITY


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Our goal is to develop a method for reversible suppression of menses through use of antiprogestins. In the current year we showed that treatment with low doses of the new generation antiprogestin, ZK 230 211 will block menses in rhesus macaques. Doses of ZK 230 211 that block menses also blocked ovulation, but these effects were reversible once treatment stopped. Studies on ovariectomized-hormone treated macaques defined a range of doses of ZK 230 211 from 0.005 mg/kg, which had minimal effects, to a full blockade of progesterone action at 0.032 mg/kg. Doses > 0.016 mg/kg blocked progesterone action and also inhibited the proliferative effects of estradiol, resulting in reduced endometrial mass and thickness. No untoward effects of ZK 230 211 were detected in the macaques. The inhibitory action of ZK 230 211 on the endometrium could be demonstrated with both systemic and local delivery of the compound and we showed that ZK 230 211 could be effectively administered with intrauterine devices (IUDs). Local (IUD) delivery of antiprogestins appears to confine action to the uterus and should avoid ovarian effects. Overall, these studies indicate that antiprogestin therapy with ZK 230 211 should provide a safe, method to suppress menstruation in women.
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In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.
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INTRODUCTION

The goal of this research is to develop a safe, reversible method of menstrual suppression through antiprogestin therapy. Antiprogestins are synthetic ligands for the progesterone (P) receptor that antagonize P action. The antiprogestins we are testing (ZK 137 316 and ZK 230 211) are new generation compounds of enhanced potency and specificity, manufactured by Schering AG, Berlin.

As a brief overview, we first tested various low doses of ZK 137 316 (years 1 and 2) and found that ZK 137 316 reversibly inhibited menstruation in cycling rhesus macaques during both short term (40 day) and long term (100 day) trials. Schering has recently provided a new antiprogestin compound ZK 230 211, that has several attributes that make it more appropriate for menses blockade therapy than ZK 137 316. First, ZK 230 211 is a much more potent antiprogestin, that appears to inhibit progesterone (P) action at doses 10 fold lower than ZK 137 316, and 100 fold lower than the classical antiprogestin, RU 486. Second, at these low doses, ZK 230 211 is a pure P antagonist, with no significant antiglucocorticoid, antiandrogen, or P agonist activity. Third, ZK 230 211 is both systemically and orally active. Moreover, ZK 230 211 can be administered through vaginal gel preparations and intrauterine devices, providing direct local administration of the compound. In year 2, we conducted preliminary dose finding trials with ZK 230 211 (0.005 mg-0.1 mg/kg). We found that a dose of ≥ 0.01 mg/kg induced frank menses in P primed monkeys, a clear indication of P blockade.

In a logical extension of this work, we have now (year 3) evaluated the potency of ZK 230 211 on endometrial growth and differentiation, and tested the ability of ZK 230 211 to block menses in naturally cycling macaques. We have further begun studies of local delivery of ZK 230 211 through steroid-releasing IUDs.

RESEARCH ACCOMPLISHED

1. Blockade of Menstruation with ZK 230 211

Methods: Animal care throughout these studies was provided by the Oregon Regional Primate Research Center (ORPRC) Division of Animal Resources, and all procedures were reviewed by the ORPRC Institutional Animal Care and Use Committee. Untreated, adult cycling rhesus monkeys were monitored for 2 menstrual cycles to document normal cycle lengths for each animal. Beginning on the day after onset of the third menstruation the animals were injected i.m. daily for 60 days with ZK 230 211 dissolved in 37.5 % Hanks Balanced Salt Solution, 37% 1,2-propanediol, and 25% ethanol (HBSSPE). Three groups (n=5 each) were treated 0.005 mg/kg, 0.016 mg/kg and 0.05 mg/kg body weight. Because we have previously shown that injection with this vehicle had no effect on hormone levels, menstrual cycles or endometrial bleeding we used the pretreatment cycles for each group to represent control cycles. Daily vaginal swabs (to detect vaginal bleeding) and daily blood samples were collected during the pretreatment cycle, the treatment period (60 days) and the recovery period until the monkeys menstruated for longer than 2 days. The monkeys were further monitored for menses during the first post-treatment cycle. The experimental design is shown in the diagram below.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment (60 days)</th>
<th>Recovery</th>
<th>Post -treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle</td>
<td>Intermenstrual Interval</td>
<td>Cycle</td>
<td></td>
</tr>
<tr>
<td>Mense</td>
<td>Mense</td>
<td>Mense</td>
<td></td>
</tr>
</tbody>
</table>
Blood samples were analyzed for concentrations of E2 and P by routine radioimmunoassay, performed by the ORPRC Hormone Assay Core [1]. Lengths of the pre-treatment menstrual cycles, treatment-induced inter-menses interval, length of the recovery period (time to return to menses) after the last injection, post-treatment menstrual cycle length and serum hormone levels were compared between treatment groups. Statistical comparison of intermenstrual interval between groups was done by analysis of variance, followed by Fisher's Protected LSD Test [2].

**Results.** No untoward effects of ZK 230 211 treatment were detected in the monkeys during the study. Effect of 60 day ZK 230 211 treatment on menstrual cycle length are presented in Figure 1. The macaques in all of the groups exhibited normal length pretreatment menstrual cycles (27.6±2.1 days). Injection with 0.005 mg/kg had no effect on menstrual cyclicity, and all the animals menstruated normally at 27.2 ± 1.0 day intervals. This dose also had no effect on post-treatment menstrual cycles. In contrast, injection with 0.016 mg and 0.05 mg ZK 230 211/kg blocked menstruation and significantly extended the intermenstrual interval in all of the animal to approximately 100 days (P<0.01; Fig 1). In these two groups, the recovery period (from the last injection to the first post-treatment menses) was 34 ± 6 days. These two doses also slightly increased the length of the post-treatment menstrual cycle to approximately 50 days (P<0.05).

All of the animals had normal patterns of E2 and P during the pretreatment cycle (Fig. 2). In addition, animals in the 0.005 mg/kg groups expressed normal menstrual cycle patterns of E2 and P throughout the treatment and recovery periods period. When macaques were treated with 0.016 mg/kg daily, a normal follicular phase pattern of E2 surges was observed, but normal luteal phase levels of P did not develop, suggesting a blockade of ovulation. Monkeys treated with 0.05 mg further failed to develop either a normal E2 surge or normal luteal phase levels of P. Despite blockade of E2 surges in this group, all of the monkeys showed normal nonsurge levels of E2 (~30-100 pg/ml) throughout the treatment period. In the two groups P levels failed to rise above 0.5 ng/ml during the treatment period, suggesting failure or absence of a functional corpus luteum. Approximately 10 days after treatment ended, both groups demonstrated rise in serum P, and a normal length luteal phase. Menses occurred following P decline at the end of this luteal phase (Fig. 2).

**Summary:** Our results indicate that treatment with 0.005 mg/kg ZK 230 211 is not sufficient to inhibit menstruation or ovarian function. In contrast, administration of doses ≥ 0.016mg/kg/day provide reversible inhibition of menses in macaques. While these doses have no untoward effects, they do inhibit ovulation and normal luteal function. This ovarian blockade ceased when treatment was stopped. Although ovulation and luteal function was blocked at these "menses-inhibiting" doses, normal non surge levels of E2 were observed, even at 0.05 mg/kg, suggesting that unwanted effects of estrogen withdrawal (e.g., bone loss, hot flushes etc.) would not occur. Overall, these effects suggest that daily administration of ZK 230 211 may provide a therapy for menses inhibition for the military woman.

2. Endometrial Inhibition with Low Doses of ZK 230 211

**Methods:** We have begun testing the effects of various doses of ZK 230 211 on endometrial thickness, mass, histology, and expression of estrogen receptor (ER), P receptor (PR) and Ki-67 antigen (a marker of cell proliferation) in ovariectomized artificially cycled macaques. The artificial cycles were induced in ovariectomized monkeys by first inserting a 3 cm E2-filled Silastic capsule for 14 days to produce an artificial follicular phase, and then after E2 priming, a 6 cm P-filled Silastic capsule was implanted for 14 days to stimulate an artificial luteal phase. Normal follicular phase levels of E2 (80-100 pg/ml) and luteal phase levels of P (4-6 ng/ml) were confirmed in serum of these animals by radioimmunoassay. All animals
completed one full artificial cycle before treatment with antiprogestin, and as expected, withdrawal of the P implant at the end of the pretreatment cycle resulted in menstruation.

The monkeys were assigned to two hormone regimens (protocols) shown below. In protocol 1, the monkeys were maintained under continuous $E_2$ stimulation for 28 days ($E_2$ alone). Beginning on day 1 of the treatment the animals were injected daily with ZK 230 211 (i.m. in HBSSPE) for the entire 28 day period. In protocol 2, the monkeys were treated with $E_2$ to create a normal 14 day artificial follicular phase and then a P implant was inserted for 14 days to create an artificial luteal phase ($E_2 + P$). In this case, the animals, were injected daily with ZK 230 211 throughout both the artificial follicular phase, and luteal phase. Three doses of ZK 230 211 were tested in each protocol: 0.005 mg, 0.016 mg, and 0.032 mg. Control animals received no ZK 230 211 (n=5). The monkeys were hysterectomized at the end of each protocol and the uterus from each animal was dissected longitudinally into equal quarters. The endometrium from one quarter was separated from the myometrium and each component weighed. The remaining endometrium was then prepared for histology, and immunocytochemistry of ER, PR and Ki-67 antigen as previously described [3;4]. Endometrial thickness was quantified with the Optimas™ image analysis software system on digital photos captured with an Optronics digital camera.

**Experimental design**

**Protocol 1**

<table>
<thead>
<tr>
<th>Pretreatment Cycle</th>
<th>Treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implant $E_2$</td>
<td>Remove P</td>
</tr>
<tr>
<td>$E_2$</td>
<td>$E_2 + P$</td>
</tr>
<tr>
<td>d 0</td>
<td>d 28/0</td>
</tr>
<tr>
<td>d 14</td>
<td>d 28</td>
</tr>
<tr>
<td>Inject ZK 230 211</td>
<td></td>
</tr>
</tbody>
</table>

**Protocol 2**

<table>
<thead>
<tr>
<th>Pretreatment Cycle</th>
<th>Treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implant $E_2$</td>
<td>Implant P</td>
</tr>
<tr>
<td>$E_2$</td>
<td>$E_2 + P$</td>
</tr>
<tr>
<td>d 0</td>
<td>d 28/0</td>
</tr>
<tr>
<td>d 14</td>
<td>d 14</td>
</tr>
<tr>
<td>Implant P</td>
<td></td>
</tr>
<tr>
<td>Inject ZK 230 211</td>
<td></td>
</tr>
</tbody>
</table>

**Results:** Table 1 shows the effect of ZK 230 211 on endometrial thickness and endometrial and myometrial mass, when administered in the presence of $E_2$ alone (Protocol 1). No statistical analysis has been conducted on these values, because data collection for this
experiment is still underway and the current sample size is small. However, the general trend is clear, compared to 28 days of $E_2$ alone, treatment with $E_2 + ZK 230-211$ resulted in a ZK dose-dependent inhibition of endometrial thickness and mass, which was similar at 0.016 and 0.032 mg/kg. Because there was no clear effect of ZK on myometrial mass or thickness, the inhibitory action appears to be endometrium specific.

Figure 3 and 4 shows photomicrographs of the endometrium of monkeys treated with various doses of ZK 230 211 under protocol 1. Treatment with $E_2$ alone for 28 days resulted in a normal proliferative endometrium marked by tubular glands, expanded stroma (Fig. 3a) and abundant mitotic cells (Fig 3e). $ZK 230 211$ at all doses reduced the abundance of mitotic cells and increased the abundance of apoptotic cells (compare Fig 3e-h). This reduction in endometrial mass was associated with an increase in stromal compaction, as seen previously with ZK 137 316 [4] (compare Fig. 3a-d). Figure 4 shows ICC staining for ER, PR, and Ki-67 antigen in control and ZK treated endometria. Strong ER and PR staining, and abundant Ki-67-positive cells were observed in animals treated for 28 days with $E_2$ alone. Treatment with $E_2 + ZK 230 211$ did not inhibit ER or PR staining. However, there was an apparent reduction in the number of Ki-67 stained epithelial cells at higher doses of ZK (compare Fig. 6k-l).

Table 2 presents endometrial and myometrial measurements from animals under protocol 2 ($E_2 + P$). Treatment with $E_2$ and then $E_2 + P$ resulted in maximal endometrial thickness and mass. However, even in the presence of $P$, $ZK 230 211$ at all doses reduced endometrial thickness compared to $E_2 + P$ controls. This ZK dose dependent reduction in mass and thickness was minimal at 0.005 mg/kg ZK 230 211 and maximal at 0.032.

Figure 5, presents micrographs of the endometrium of monkeys treated with various doses of ZK 230 211 under protocol 2 ($E_2 + P$). Treatment with $E_2$ and then $E_2 + P$ resulted in a secretory phase endometrium marked by sacculated glands, hypertrophied stroma (Fig. 5a) and secretory glandular epithelium (Fig 5e). At the lowest dose (0.005mg/kg) ZK 230 211 had no clear effect of P-stimulated differentiation. In contrast, treatment with ZK 230 211 at dose of $\leq 0.016$ mg/kg $ZK 230 211$ resulted in a blockade of $P$ action and a generally proliferative type morphology. (Compare Fig. 5a-e). As expected, treatment with $E_2 + P$ resulted in a down regulation of ER, PR, and Ki-67 antigen (Fig. 6) in the glandular epithelium (but some staining was still observed in the stroma). Cotreatment with 0.005 mg/kg ZK had little effect on $P$ action. However, higher doses (0.016 and 0.032) resulted in a dose-dependent increase in both ER, PR and Ki-67 staining indicating a full blockade of $P$ action. Despite the apparent increase in Ki-67 staining in $E_2 + P + ZK$-treated animals, mitotic counts (not shown) indicated a decrease in epithelial cell proliferation after ZK-treatment, similar to observations reported after other antiprogestin treatments [4].

Summary: These results document a full range of effects of ZK 230 211 on $P$ action, from minimal effects at 0.005 mg/kg to full blockade of $P$ action, at $\geq 0.016$ mg in all animals. In addition, ZK at these higher doses did not block $E_2$ action on ER and PR regulation, but did appear to decrease epithelial cell proliferation. This suggests that at these doses, ZK 230 211 can block $P$ action, but also block unwanted $E_2$ stimulated cell proliferation.

3. Local Delivery of Antiprogestins via Intrauterine Devices

Our studies suggest that chronic, systemic antiprogestin therapy can block the key endometrial effects of both estrogens and progestins, suppress endometrial growth and development and inhibit menstrual bleeding. Unfortunately, systemic doses of antiprogestins that block menses (e.g., 137 316, and 230 211) may also be antiovulatory. The goal of this study was to develop an intrauterine device in rhesus macaques to deliver significant concentrations of ZK 230 211 (to block the endometrium and menstruation) without producing significant systemic levels.
Methods: To determine the appropriate size IUD for use in macaques, we measured uterine circumference, distance from internal os to fundus, and cervical length during laparotomy and also after hysterectomy. Based on these measurements, Leiras OY, Finland, a subsidiary of Schering AG, manufactured antiprogestin-containing IUDs to our size and dose specification. The IUDs consisted of straight Silastic tubes with an attached thread to facilitate anchoring the IUD. IUDs with two different release rates were made. One released a high dose (26-30.2 μg/day) and the other a low dose (3.3-4.5 μg/day) of ZK 230 211.

We originally considered stumptail macaques (Macaca arctoides) as the most suitable species for this project because it had been reported that they have a cervix that is straight compared to the S-shaped cervix of other common laboratory macaques. We therefore, recruited six stumptail macaques to initiate this study. However, we had great difficulty in cannulating the cervix of the stumptails and discovered that it was not possible to reliably pass IUDs into the uterus in this species. We therefore abandoned the transcervical approach and developed a surgical technique to place the IUDs in the uterine lumen by hysterotomy.

This experiment was designed to test whether ZK 230 211-releasing IUDs could inhibit the endometrial effects of systemic P in artificially cycled macaques. Three ovariectomized stumptail macaques were first treated with E2-filled Silastic implants for 14 days and then with E2 plus P implants for 14 days to induce artificial menstrual cycles. On the 14th day of P treatment, (i.e., on the 28th day of the artificial cycle) IUDs were inserted into the uterine lumen by hysterotomy. We used a blank IUD (control), a high dose ZK 230 211-releasing IUD, and a low dose ZK 230 211-releasing IUD.

Both the low and high doses of the ZK-filled IUDs induced menstruation within three days of inserting the IUDs, while there was no detectable bleeding in the animal treated with the blank IUD (control). This indicated that the amount of antiprogestin produced locally by both the high and low dose IUDs was sufficient to prevent systemic P from maintaining the endometrium in a progestational state. Because the control implants did not induce bleeding, it appeared there were no major effects of hysterotomy or insertion of the IUD on the progestational state of the endometrium.

Seven days after the IUDs were installed, with the E2 implants left in place, the P implants were removed for 14 days and then replaced for 14 days to create one full artificial cycle. At the end of the cycle, laparotomies were performed and a wedge biopsy of the uterine wall of each animal was made. Tissues were processed for histology and immunocytochemistry.

Examination of these biopsies showed that, compared to the control IUDs, the ZK 230 211 IUDs induced a dramatic, dose dependent inhibition of endometrial development. The endometrium exposed to the blank IUD showed no major differences from a typical progestational endometrium, except that the amount of endometrial tissue was somewhat less than would be expected at the end of a normal cycle, and there was a modest infiltration of leucocytes in the upper functionalis. This resembled a foreign body reaction typically seen with IUDs. These results indicate that surgically installed blank IUDs are appropriate controls for the proposed studies. In contrast, the antiprogestin IUDs caused a severe compaction of the stroma and an inhibition of the effects of P on both glandular sacculcation and spiral artery development similar to that seen after systemic treatment (above). Immunohistochemical staining for ER and PR revealed that in the control IUD samples, ER and PR staining was weak in the glands and detectable in the stroma, but both the low and high dose antiprogestin IUDs greatly increased ER and PR staining in both these cell types. This indicates that local antiprogestin can inhibit the well known ability of systemic P to downregulate glandular and stromal ER and PR.

The animals with the blank IUDs menstruated normally when the P implant was removed, as expected. The animals with the antiprogestin IUDs did not bleed when the P implant was removed. The failure to bleed when P was withdrawn suggested that while the ZK
230 211-releasing IUDs were in place, any progestational effect of P to prime the endometrium for bleeding or menses was also blocked. This is suggestive evidence that antiprogestin IUDs can block both P withdrawal bleeding and menstruation in cycling macaques and women.

We examined the oviductal fimbriae to evaluate the systemic effects, if any, of the antiprogestin. In all animals, control as well as antiprogestin IUD treated, the oviductal epithelium was atrophied, nonsecretory and deciliated, classic signs of P action in macaques. There was no evidence that the antiprogestin had reached levels in the circulation adequate to inhibit P action in the oviduct. If P action had been inhibited in the oviduct, unopposed estrogen would have stimulated cellular hypertrophy, ciliogenesis and secretion. We conclude that the antiprogestin released by these IUDs was confined to the endometrium and that all the antiendometrial effects we observed were due to the action of local, not systemic, ZK 230 211.

CONCLUSIONS

We have now conducted menses suppression trials with a wide range of doses of ZK 230 211 in rhesus monkeys and have identified a range of doses that will fully block frank menses in intact cycling animals. We have further shown that this treatment is fully reversible and that the monkeys regain normal menstrual cyclicity beginning with their first menstruation following treatment. However, doses of ZK 230 211 that inhibit menses also blocked ovulation. In spayed-hormone treated macaques, menses inhibiting doses of ZK 230 211 also inhibited endometrial development, blocking P action, but allowing some E2 action. This suggests that effective doses of antiprogestin that block endometrial thickness are very close to the doses that block ovulation. Therefore, it would be desirable to develop a therapy that had no effects on ovarian physiology. We have now shown ZK 230 211 can be administered via steroid releasing IUD. Our future studies will continue to focus on novel modes of local delivery of ZK 230 211 and on the mechanism of action of antiprogestins in the macaque uterus. In summary, our studies indicate that antiprogestin treatment should provide a safe, reversible therapy to suppress menstruation in women.

Literature Cited


Table 1. Endometrial thickness, and myometrial and endometrial weight in $E_2 + ZK 230 211$-treated macaques (Protocol 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$E_2$ alone</th>
<th>$E_2 + 0.005$ mg ZK</th>
<th>$E_2 + 0.016$ mg ZK</th>
<th>$E_2 + 0.032$ mg ZK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial Thickness (mm)</td>
<td>3.4±0.4 (n=5)</td>
<td>2.4±0.3 (n=3)</td>
<td>2.1±0.2 (n=2)</td>
<td>1.9±0.8 (n=2)</td>
</tr>
<tr>
<td>Myometrial Mass (g)</td>
<td>1.49±0.20 (n=5)</td>
<td>1.28±0.44 (n=3)</td>
<td>0.77±0.36 (n=2)</td>
<td>1.04±0.38 (n=2)</td>
</tr>
<tr>
<td>Endometrial Mass (g)</td>
<td>0.35±0.03 (n=5)</td>
<td>0.15±0.06 (n=3)</td>
<td>0.05±0.01 (n=2)</td>
<td>0.07±0.01 (n=2)</td>
</tr>
</tbody>
</table>

Values represent mean±SE. Endometrial thickness was measured at 2.5X with Optimas™ image analysis software on digital photos of 2-3 histological sections from each animal, captured with an Optronics digital camera. Endometrial and myometrial mass values represent the mass from 1/4 of the uterus of each animal.

Table 2. Endometrial thickness, and myometrial and endometrial weight $E_2 + P + ZK$-treated macaques (Protocol 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$E_2 + P$</th>
<th>$E_2 + P + 0.005$ mg ZK</th>
<th>$E_2 + P + 0.016$ mg ZK</th>
<th>$E_2 + P + 0.032$ mg ZK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial Thickness (mm)</td>
<td>4.5±0.55 (n=5)</td>
<td>3.01±0.63 (n=3)</td>
<td>2.2±0.28 (n=2)</td>
<td>2.0±0.22 (n=2)</td>
</tr>
<tr>
<td>Myometrial Mass (g)</td>
<td>1.15±0.15 (n=5)</td>
<td>1.46±0.38 (n=3)</td>
<td>1.19±0.19 (n=2)</td>
<td>0.8±0.11 (n=2)</td>
</tr>
<tr>
<td>Endometrial Mass (g)</td>
<td>0.41±0.01 (n=5)</td>
<td>0.18±0.07 (n=3)</td>
<td>0.11±0.04 (n=2)</td>
<td>0.06±0.04 (n=2)</td>
</tr>
</tbody>
</table>

Values represent mean±SE. Endometrial thickness was measured at 2.5X with Optimas™ image analysis software on digital photos of 2-3 histological sections from each animal, captured with an Optronics digital camera. Endometrial and myometrial mass values represent the mass from 1/4 of the uterus of each animal.
Figure 1. Length (days) of the pretreatment menstrual cycle, treatment-induced intermenses interval (Treatment), and posttreatment cycle, in intact macaques injected daily with ZK 230 211. Values represent mean ± SE (n=5). Bars with different superscripts are statistically different (P<0.05).
Figure 2. Effect of ZK 230 211 treatment on $E_2$ and $P$ levels in intact rhesus macaques. Hatched box depicts the treatment period at each dose. Values represent mean $\pm$ SE (n=5). Treatment with 0.005 mg/kg ZK had no effect on cyclic levels steroid hormones. Treatment with 0.016 mg/kg blocked luteal phase $P$, and 0.05 mg/kg further inhibited $E_2$ surges.
Figure 3. Photographs of GMA sections showing the effects of E2 + ZK 230 211 on endometrial (Endo) thickness and histology. 3 a-d, show low power photographs, a line has been drawn at the endometrial -myometrial (Myo) border. 3 e-h show high power micrographs. Arrows indicate mitotic cells (M) and apoptotic cells (A) in the glandular epithelium.
<table>
<thead>
<tr>
<th></th>
<th>E₂ alone</th>
<th>E₂ + 0.005 mg ZK</th>
<th>E₂ + 0.016 mg ZK</th>
<th>E₂ + 0.032 mg ZK</th>
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<td>Ki-67</td>
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Figure 4. Photomicrographs of estrogen receptor (ER) progesterone receptor (PR) and Ki-67 immunocytochemistry after E₂ + ZK 230 211 treatments. S = stroma; G = glands. Arrows show Ki-67-positive cells.
Figure 5. Photographs of GMA sections showing the effects of E2 + P + ZK 230 211 on endometrial (Endo) thickness and histology. 5 a-d, show low power photographs, a line has been drawn at the endometrial - myometrial (Myo) border. 5 e-h show high power micrographs. Treatment with ZK inhibited P-induced glandular development (compare e-h).
MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for reports on the enclosed list be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

[Signature]

PHILIS M. RINEHART
Deputy Chief of Staff for Information Management

Encl