Drospirenone: A Novel Progestogen with Antimineralocorticoid and Antiandrogenic Activity

Pharmacological Characterization in Animal Models

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Drospirenone (ZK 30595; 6β, 7β, 15β, 16β-dimethylen-3-oxo-17α-pregn-4-ene-21, 17-carbo-lactone) is a novel progestogen under clinical development. Potential applications include oral contraception, hormone replacement therapy and treatment of hormonal disorders.

Drospirenone is characterized by a pharmacodynamic profile very closely related to that of progesterone. The progestogenic activity of drospirenone has been analysed in a variety of animal models. The compound efficiently promotes the maintenance of pregnancy in rats, inhibits ovulation in rats and stimulates endometrial transformation in the rabbit. Furthermore, drospirenone shows potent antigonadotropic, i.e. testosterone-lowering, activity in male cynomolgus monkeys. The progestogenic potency of drospirenone was found to be in the range of that of norethisterone acetate or cyproterone acetate.

Like progesterone, drospirenone has been shown to have an antimineralocorticoid effect in rats and humans. It has now been demonstrated that the compound has a long-lasting natriuretic activity in rats on administration of a daily dose of 10 mg s.c. for three weeks. Under identical conditions, spironolactone, a widely-used antimineralocorticoid, becomes ineffective after the initial treatment phase.

Drospirenone exhibits antiandrogenic activity in castrated, testosterone-substituted male rats as shown by dose-dependent inhibition of accessory sex organ growth (prostate, seminal vesicles). In this model, the potency of drospirenone was found to be about one-third that of cyproterone acetate.

The compound is devoid of androgenic, estrogenic, glucocorticoid and antiglucocorticoid activity.

Possible drug interaction between drospirenone and ethinylestradiol (EE) was also investigated. EE did not interfere with either the progestogenic or the antimineralocorticoid activity of drospirenone.

In conclusion, drospirenone represents a novel type of synthetic progestogen since it combines potent progestogenic characteristics with antimineralocorticoid and antiandrogenic activity. Thus, the pharmacological profile of drospirenone is more closely related to that of the natural hormone progesterone than is that of any other synthetic progestogen in use today. Therefore, drospirenone is anticipated to give rise to a number of additional health benefits both for users of oral contraceptives and hormone replacement therapy recipients. CONTRACEPTION 1995;51:99–110

KEY WORDS: progestogen, antiandrogen, antimineralocorticoid, progesterone, oral contraception, hormone replacement therapy

Introduction

Progesterone and the synthetic progestogens usually display a mixture of distinct hormonal and/or antihormonal activities *in vivo*. Depending on their structure, progestogens may also have estrogenic (norethynodrel), androgenic (norethisterone, levonorgestrel) or antiandrogenic (cyproterone acetate, chlormadinone acetate) effects (see Ref. 1). Besides its progestogenic activity, progesterone gives rise to antimineralocorticoid[2–8] and antiandrogenic effects *in vivo*.[9–11] The antiandrogenic activity of progesterone has also been demonstrated in a transactivation assay employing stable transfection of a cell line with the androgen receptor and a reporter gene (Ref. 12 and Fuhrmann et al., unpublished data).

Most synthetic progestogens presently used in oral contraception and hormone therapy differ from progesterone in that they have no antiandrogenic or antimineralocorticoid activity. Exceptions to this are cyproterone acetate and chlormadinone acetate with their antiandrogenic properties[13] and gestodene, which has a weak antimineralocorticoid effect.[14,15]

Drospirenone[16] is a novel type of progestogen: the compound represents the first potent synthetic progestogen to exhibit both antiandrogenic and an-

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timineralocorticoid activity at pharmacologically relevant dose levels. Drospirenone's antimineralocorticoid effects have been demonstrated in rats[14] and in humans.[17] The compound is devoid of any additional steroid agonistic or antagonistic activity. The binding affinity to steroid hormone receptors has been investigated (Ref. 18; Fritzemeier, unpublished data). The relative binding affinity (RBA) to the progesterone receptor is 42% (progesterone = 100%), to the androgen receptor 0.6% (dihydrotestosterone = 100%) and to the mineralocorticoid receptor 100% (aldosterone = 100%).

It is discussed that, due to its antimineralocorticoid activity, drospirenone can be expected to minimize certain estrogen-induced side effects in women and to give rise to a number of additional health benefits on clinical application.

Materials and Methods

Animals

Rats (HAN:WIST) were obtained from TZH Schering AG, Berlin, unless otherwise indicated. Rabbits came from Med. Versuchstierzucht SAVO, Germany. Cynomolgus monkeys were purchased from Hazelton Laboratories (Germany). Animals were kept under standard conditions unless otherwise indicated.

Chemicals

Steroids used for the experiments were synthesized at the Dept. of Medical Chemistry, Schering AG, Berlin (drospirenone; spironolactone; progesterone; cyproterone acetate; levonorgestrel; testosterone propionate; mifepristone; dexamethasone; estradiol; ethinylestradiol). All other chemicals were purchased in the highest purity available. One tenth ml (mice) or 0.2 ml (rats) benzyl benzoate/ castor oil 1:5 (v/v) was used as a vehicle for s.c. drug application. For p.o. application, rats were treated with 0.5 ml of a 0.085% Myrj solution in a 0.9% NaCl vehicle.

Methods

Progestogenic Activity of Drospirenone

ENDOMETRIAL TRANSFORMATION TEST IN THE RABBIT. Juvenile New Zealand white rabbits weighing about 1.2 kg were ovariectomized and randomly distributed into groups of 6 to 8 animals. Beginning 7 days later, they were treated s.c. or p.o. for 6 consecutive days with 5 μg/animal 17β-estradiol. This was followed by 5 days' treatment with a daily dose of 0.1, 0.3, 1 mg/animal drospirenone; 0.01, 0.03, 0.1 mg/animal levonorgestrel; or 3, 10, 30 mg/animal spironolactone as reference. The animals were sacrificed on the day following completion of treatment. Uterine horns were dissected and examined histologically.

Progestational (secretory) transformation of the endometrium was assessed in histological preparations according to a modified McPhail scale (rating grades 1–4; 1 = no action; 4 = complete transformation; see Ref. 19). The dose that results in a McPhail value of 1.5 as group average was considered to represent the threshold value.

INHIBITION OF OVULATION IN THE CYCLIC RAT. Rats with regular 4-day cycles were treated s.c. for 4 consecutive days with drospirenone (0.3, 1, 3, 10 mg/animal/day) or levonorgestrel (0.003, 0.01, 0.03, 0.1 mg/animal/day; 6 animals per dose), beginning in metestrus. Unilateral ovariectomy and tubectomy were carried out under ether narcosis on the fourth day. Crush preparations were prepared from the tubes and examined by light microscopy for the presence of oocytes. On the fifth day, the animals were sacrificed, the remaining tube was removed and investigated as described above. The percentage of animals in which ovulation was inhibited on the fourth and/or fifth day was determined in the individual dosage groups.

MAINTENANCE OF PREGNANCY IN OVARIECTOMIZED RATS. Female rats (Moellegard Breeding Center Ltd., Denmark) weighing 200–230 g were mated. The presence of sperm in vaginal smears was considered day 1 of pregnancy. The animals were ovariectomized on day 8 p.c. and treated with estrone (1 μg/animal s.c.), as well as either drospirenone or cyproterone acetate. The progestogens were given either s.c. or p.o. Subcutaneously applied doses were 0.1, 1, 3 and 10 mg/animal/day (10 animals per dose). The oral doses were 1 × 3 mg every 24 hrs, 2 × 1.5 mg every 12 hrs, 3 × 1 mg every 8 hrs or 6 × 0.5 mg every 4 hrs in 0.5 ml vehicle. Treatment was started on day 8 p.c. 2 hrs before ovariectomy and continued for 4 consecutive days (days 8—11 p.c.). Animals were sacrificed one day after the last treatment (day 12 p.c.). The degree of maintenance was calculated by dividing the number of live fetuses by the total number of implantation sites in the left uterine horn (given in %). Fetuses with beating hearts were regarded as alive. The absence of implantation sites (castrated controls) was defined as 0% pregnancy maintenance.

ANTIGONADOTROPIC EFFECT IN MALE CYNOMOLGUS MONKEYS. Male cynomolgus monkeys (*Macaca fascicularis*) were treated with 4 mg drospirenone/kg/day p.o. or 40 mg spironolactone/kg/day p.o. on days

5, 7, 10, 12 and 14 (5 animals/group) of the experiment. Blood samples were drawn before (days 1 and 3) and 24 hrs after each treatment. Serum testosterone was determined by radioimmunoassay (S. Hasan, unpublished).

Anabolic/Androgen Test in the Castrated Rat

Juvenile male rats (HAN:WIST) weighing about 100 g were castrated. Beginning seven days after castration, they received s.c. injections of 10 mg drospirenone/animal/day or 0.1 mg testosterone propionate/animal/day within a two-week period (n = 6 animals/group). Animals were sacrificed on the day after the last treatment and the fresh weights of seminal vesicles, prostate and M. levator ani were determined. For evaluation, organ weights were normalized to 100 g body weight. The mean value and standard deviation were calculated for each group. The differences between the groups were tested for statistical significance by multiple variance analysis.

Antiandrogenic Activity of Drospirenone

ANTIANDROGEN TEST IN THE CASTRATED RAT. Juvenile male rats (HAN:WIST) weighing about 100 g were castrated. Beginning 7 days later, groups of 6 animals were treated s.c with drospirenone or cyproterone acetate as a reference compound at doses of 0.1, 0.3, 1, 3 or 10 mg/animal/day together with 0.1 mg testosterone propionate (TP)/animal/day for 7 days. Controls received only the vehicle or TP. One day after the last treatment, the animals were sacrificed. The weights of the seminal vesicles and prostate were determined. In addition, the weight of the adrenal glands and the body weight were recorded. All organ weights were normalized to mg/100 g body weight. Data were evaluated by plotting the percentage inhibition of organ weight against the corresponding dose, whereby the TP controls were set at 100%. Statistical evaluation was carried out with a least significance (LSD) test to compare the TP control with the various treatment groups. In case of significance, this test was followed by pair-wise t-tests using the mean square for error.

SEXUAL DIFFERENTIATION (FEMINIZATION/VIRILIZATION TEST). Pregnant rats were treated s.c. with drospirenone (0.1, 0.3, 1, 3 or 10 mg/animal/day) or cyproterone acetate (0.1, 0.3, 1, 3 or 10 mg/animal/day) on days 17 to 20 p.c. Day 1 of pregnancy was identified by the presence of sperm in the vaginal plug. Six mother animals were treated in each dosage group. The vehicle control group consisted of 12 animals. The animals were sacrificed on day 23 p.c. For morphological evaluation the fetuses were fixed in Bouin solution. For histological studies, a 2–3 mm slice was cut out of the middle of the fetuses and embedded in paraffin. Sexual differentiation was assessed in serial sections through the internal sexual organs as well as through the urogenital sinus (UGS) after hematoxylin-eosin staining. Qualitative parameters taken were the type of perineal region, the presence of ventral glandar lamella and the presence of prostate anlagen. Quantitative parameters were the anogenital distance and the length of the UGS which were evaluated with a VIDEOPLAN system (MOP AM 02/HP9815A; Kontron, Japan). Mean and standard deviation were calculated for each group. In addition, multiple variance analysis was performed.

Evaluation of Long-Term Antimineralocorticoid Activity in Female Rats

Rats weighing 210–240 g were ovariectomized on day 1. From day 1 to 30 they were fed a low-sodium diet containing 0.014% sodium (Altromin 1324; Altromin GmbH, Germany). From day 9 to 29, groups of 6 animals were treated s.c. with drospirenone or spironolactone (1, 3, 10 mg/animal/day). Ten animals treated with vehicle served as controls. Urine was collected for 24 hrs on days 9, 10, 12, 16, 19, 23, 26 and 30. Thereafter, the animals were sacrificed. Blood samples from the retrobulbar plexus were taken under ether narcosis at 8.00 a.m. on day 5 (pretreatment) and day 30 (one day after the last treatment). Then, urine concentrations of sodium and potassium ions were determined by flame photometry. Serum aldosterone was determined with a commercial radioimmunoassay (Biermann, Germany). Treatment effects were evaluated against the control with the Dunnet test. In cases of overall significant difference, pair-wise comparison between individual treatment and control groups was carried out with the Wilcoxon test. Sodium and urine excretion was evaluated by area under the curve (AUC) analysis (Kruskal-Wallis test).

Glucocorticoid/Antiglucocorticoid Activity in Rats

ADRENAL WEIGHT STIMULATION TEST IN THE RAT. Male SPF-Wistar rats (Schering AG, Berlin) weighing 240 g were treated with 5 mg drospirenone/animal p.o. or s.c. or vehicle on 36 days within a six-week period (n = 7 to 10 animals/group). The animals were sacrificed on the day after the last treatment and the adrenals were dissected. Their fresh weight was determined and normalized to 100 g body weight. For

statistical evaluation, multiple variance analysis was performed.

THYMOLYTIC/ANTITHYMOLYTIC ACTIVITY TEST. Juvenile male rats (HAN:WIST) weighing 100–130 g were obtained from Moellegard Breeding Center (Denmark). Animals were adrenalectomized on day 1 of the experiment and randomly assigned to the different dose regimens in groups of 7 to 10. To evaluate thymolytic effects, the animals were treated s.c. on days 6 to 9 with drospirenone (10 mg/animal/day), mifepristone (RU 486; 10 mg/animal/day), dexamethasone (0.01 mg/animal/day) or vehicle alone. Antithymolytic effects were evaluated by s.c. application of dexamethasone (0.01 mg/animal/day) and either the vehicle or one of the test compounds (drospirenone, mifepristone or cyproterone acetate at doses of 1, 3 or 10 mg/animal/day) on days 6 to 9. The animals were sacrificed on day 10. Thymus wet weights were determined and normalized to 100 g body weight. Thymolysis experiments were evaluated by calculating the percentage suppression of the thymus weight (control = 100%). Antithymolysis experiments were evaluated by calculating the drug-induced inhibition of the dexamethasone-induced thymus weight suppression (given in %). Dexamethasone-induced thymus weight suppression was regarded as 100%. For statistical analysis, the 95% confidence intervals were calculated using Fjeller's theorem.

Estrogenic Activity in Rats

Rats (HAN:WIST; Moellegard Breeding Center, Denmark) weighing between 200 and 230 g were ovariectomized on day 1. On day 10, the animals received a single dose of drospirenone (10 mg/animal) or 17β-estradiol (0.1 μg/animal) (n = 6 animals/group). Vaginal smears were investigated by light microscopy 48, 54 and 72 hrs after treatment. The animals were sacrificed immediately thereafter. Autopsy was performed and uterine wet and dry weight was determined.

Investigations into the Hormonal Interaction of Drospirenone and Ethinylestradiol

MAINTENANCE OF PREGNANCY IN RATS S.C. The method was identical to the one described under 1c, except that ethinylestradiol instead of estrone was applied to the animals at doses between 0.01 and 3 μg/animal/day.

ALDOSTERONE ANTAGONISTIC EFFECT OF DROSPIRENONE IN COMBINATION WITH ETHINYLESTRADIOL. Male or female rats (HAN:WIST; 140–160 g) were adrenalectomized. Five days later they were treated s.c. with drospirenone (0.5, 1, 2 mg/animal) or a combination of drospirenone and ethinylestradiol (1 or 10 μg/animal; n = 9–10 animals/group). One hour after application, a 15-hr infusion of a NaCl/glucose solution (3 ml/animal/hr) was started with or without the addition of aldosterone (1 μg/kg/hr). Urine fractions were collected as described in an earlier publication[1] and analysed for volume and for Na^+ and K^+ content by flame photometry.

Results

Investigations into the Progestogenic Activity of Drospirenone

The progestogenic potency of drospirenone was analysed and compared to that of other progestogens in a variety of animal models in different species.

ENDOMETRIAL TRANSFORMATION TEST IN THE RABBIT. Following subcutaneous and oral administration of a daily dose of 0.3 mg drospirenone/ animal (corresponding to approx. 0.25 mg/kg/day) over 5 days, a marked transformation of the endometrium was detected.[18]

In the dose range from 0.1 to 0.3 mg/animal s.c. (corresponding to approx. 0.008 to 0.25 mg/kg/day s.c.), endometrial reactions occurred at the threshold level (McPhail value of 1.5; see Ref. 19). Thus, the progestogenic potency of drospirenone in the rabbit is in the range of that of norethisterone acetate (see Ref. 20). The relative progestational potency of drospirenone in comparison to that of various other progestogens is given in Table 1.

INHIBITION OF OVULATION IN RATS. Upon s.c. application to rats, drospirenone inhibited spontaneous ovulation in the rat with an ID_{50} in the dose range of 0.3 to 1.0 mg/animal/day (corresponding to approx 2.1 to 7.0 mg/kg/day; see Table 2). This suggests that the

Table 1. Efficacy of various progestogens in the endometrial transformation test in the rabbit

Substance	Threshold Dose (mg/animal s.c.)
Drospirenone	0.1–0.3
Levonorgestrel	0.01–0.03
Norethisterone acetate*	0.03–0.1
Cyproterone acetate*	0.003–0.01
Progesterone*	~0.5
Spironolactone	10–20

Ovariectomized juvenile rabbits were treated with estrone and different doses of the various progestogens. Histological evaluation was performed as described under Methods. n = 6 to 8 animals/group; *: taken from Ref. 20.

Table 2. Inhibition of ovulation in the rat

Substance	ED_{50} (mg/animal s.c.)
Drospirenone	0.3–1.0
Levonorgestrel	0.01–0.03
Norethisterone acetate*	~0.3
Cyproterone acetate*	1–3
Progesterone*	~3
Spironolactone	not tested

Groups of animals with regular cycles were treated with different doses of drospirenone, beginning in metestrus. Fallopian tubes were inspected for the presence of oocytes on the fourth (unilateral ovariectomy) and fifth day (second ovary) of treatment. The absence of oocytes on both day 4 and 5 was regarded as inhibited ovulation. n = 6 animals/group; *: taken from Ref. 20.

antiovulatory potency of drospirenone is of the same order as that of norethisterone acetate (see Ref. 20). Qualitatively similar results have been obtained in mice.

Upon p.o. application to cyclic rats, drospirenone inhibited ovulation at doses above 0.3 mg/ animal/ day, the half maximal effect being reached at 1 mg/ animal/day. Drospirenone is about a third as potent as cyproterone acetate (CPA).

MAINTENANCE OF PREGNANCY IN RATS. Progestogen substitution in combination with an appropriate dose of estrone leads to maintenance of an existing pregnancy in ovariectomized rats.

The pregnancy maintenance rate in rats was 95.7% with a 4-day subcutaneous application of 10 mg drospirenone (corresponding to about 50 mg/kg/day s.c.). In this test, drospirenone was as effective as subcutaneously administered cyproterone acetate (see Figure 1 and Ref. 10). Qualitatively similar results have been obtained in mice.

After p.o. application to ovariectomized pregnant rats, drospirenone also efficiently maintained pregnancy (82.9% maintenance) with a split dose of 0.5 mg/animal 6 times a day. Application of a single dose (1 × 3 mg/animal/day) had no effect. This is presumably due to the short half-life but high bioavailability of the compound, which has been demonstrated in rats (not shown).

ANTIGONADOTROPIC EFFECT OF DROSPIRENONE IN ADULT MALE CYNOMOLGUS MONKEYS. In cynomolgus monkeys, orally applied drospirenone (4 mg/kg/day on five days) showed strong antigonadotropic activity. There was a significant reduction of serum testosterone (Figure 2) and LH levels (not shown) as compared to vehicle controls. In this respect, drospirenone was considerably more potent than spironolactone at a dose of 40 mg/kg/day p.o. This result clearly demon-

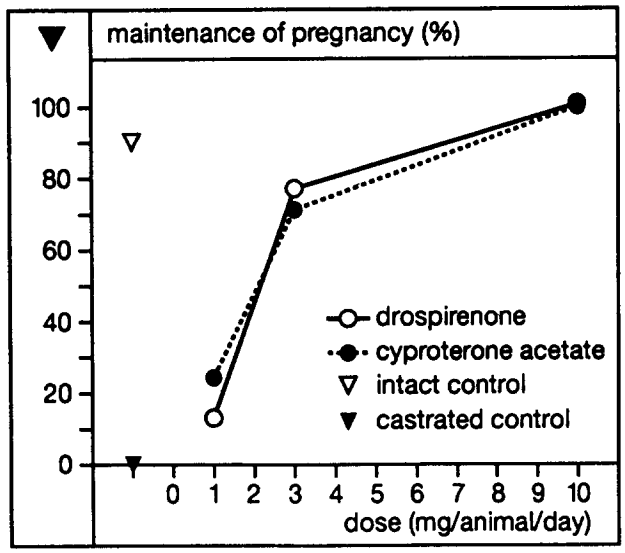


Figure 1. Maintenance of pregnancy in ovariectomized rats. Pregnant rats were ovariectomized and treated s.c. for four consecutive days (days 8 to 11 of pregnancy) with 1 μg estrone and various doses of drospirenone or cyproterone acetate. Pregnancy maintenance was expressed as the percentage of live fetuses as compared to the number of implantation sites in the left uterine horn; n = 6 animals/ group.

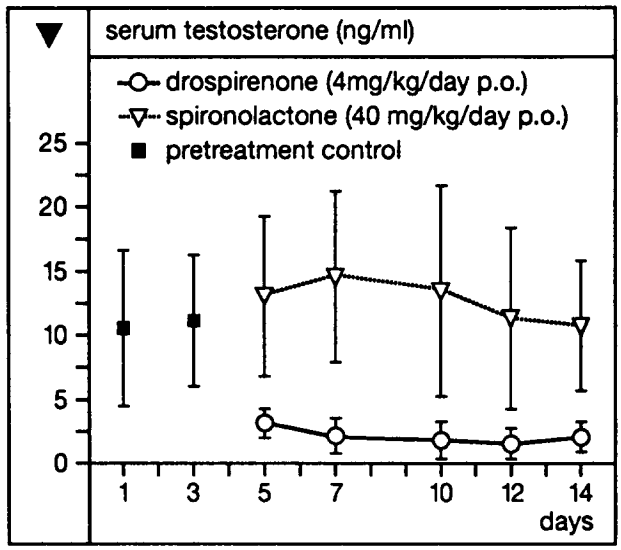


Figure 2. Antigonadotropic effect (testosterone reduction) of drospirenone and spironolactone in mature male cynomolgus monkeys. Animals were treated p.o. with drospirenone or spironolactone on days 5, 7, 10, 12, and 14. Blood samples were drawn 24 hrs after the foregoing treatment. Serum testosterone levels were determined by radioimmunoassay. Pretreatment values were obtained on days 1 and 3; n = 5 animals/group.

strates that the antigonadotropic potency of drospirenone is distinctly higher than that of spironolactone.

Anabolic/Androgenic Effect in the Rat

Castrated male rats were treated with drospirenone (10 mg/animal/ day, corresponding to about 100 mg/kg/day) for 12 days (Table 3). The treatment had no significant effect on the weight of the seminal vesicles and prostate. The reference substance testosterone propionate caused a marked increase in the weight of the accessory sex organs (seminal vesicles, prostate) at a dose of 0.1 mg/animal/day s.c. (corresponding to approx. 1.0 mg/kg/day s.c.). Thus, drospirenone did not show any androgenic properties in rats.

Antiandrogenic Activity of Drospirenone

ANTIANDROGENIC EFFECT IN CASTRATED, TESTOSTERONE-SUBSTITUTED RATS. Androgen substitution maintains the function of the accessory sex glands in castrated animals in a dose-dependent manner. Substances with antiandrogenic properties inhibit this effect.

Like cyproterone acetate, drospirenone (0.1 to 10 mg/animal/day s.c. corresponding to about 1 to 100 mg/kg/day administered for 7 days) caused a distinct dose-dependent inhibition of the growth of the seminal vesicles and the prostate (Figures 3a and b) induced by concurrent administration of testosterone propionate (0.1 mg/animal/day s.c.). In this test, drospirenone was about a third as antiandrogenic as cyproterone acetate.

Similarly, upon p.o. application to castrated and testosterone propionate-treated male rats, both cyproterone acetate and drospirenone induced a dose-dependent reduction of seminal vesicle and prostate weight (data not shown). At the highest dose tested (10 mg/animal/day), cyproterone acetate and drospirenone inhibited the testosterone-stimulated growth of seminal vesicles by 93.8% and 79.6%, respectively. Prostate growth was inhibited by 87.0% and 74.5%, respectively.

EFFECT ON SEXUAL DIFFERENTIATION (FEMINIZATION/VIRILIZATION TEST IN THE RAT). Treatment of pregnant animals with androgenic substances in the vulnerable phase of sexual differentiation leads to development of a number of internal and external genital organs in a masculine direction in female fetuses (virilization). In male fetuses, on the other hand, exposure to antiandrogens which overcome the physiological function of testicular androgens leads to female differentiation of external and internal genitalia. Most sensitive to hormone effects are the external genitalia and the urogenital sinus (UGS; Ref. 14).

Investigation of male fetuses showed that subcutaneous administration of drospirenone to pregnant rats (3 and 10 mg/animal/ day from the 17th to the 20th day of pregnancy) led to a decrease in anogenital distance and to a shortening of the UGS (Table 4). CPA (3 and 10 mg/animal/day) had a more pronounced effect on the anogenital distance and a significant effect on the UGS length was already found at a dose of 1 mg/animal/day. Drospirenone and CPA were also observed to have a feminizing effect on the prostate anlage, the perineal region and the ventral glandar lamella; CPA proved to be more potent in this respect. No effect on the internal genitalia (gonads, gonoducts, descensus testis) was found with either compound. In conclusion, the feminizing activity of drospirenone on male rat fetuses was a third to a tenth as pronounced as that of CPA, depending on the target organ.

Neither drospirenone nor cyproterone acetate had any masculinizing effect on female fetuses as the two

Table 3. The effect of drospirenone and testosterone propionate on the weight of androgen-dependent accessory sex organs in castrated male rats (anabolic/androgen test, s.c.)

	Drospirenone	Testosterone Propionate	Controls (Vehicle)
Dose (mg/animal/day)	10.0	0.1	—
Body weight (g)			
Start	100 ± 1.2	99 ± 0.8	99 ± 2.0
End	163 ± 1.7	183 ± 4.0	71 ± 2.3
Organ weight (mg/100 g BW) $\bar{x} \pm s\bar{x}$			
Seminal vesicles	6 ± 0.2	86 ± 5.5*	4 ± 0.2
Prostate	16 ± 0.8	125 ± 9.5*	12 ± 0.9
Adrenals	30 ± 0.7	41 ± 6.6	36 ± 1.3

Juvenile male rats were castrated. Beginning 7 days later, they received s.c. injections of 10 mg drospirenone or 0.1 mg testosterone propionate 12 times within 14 days. The weight of the accessory sex organs and the adrenals was determined and normalized to 100 g body weight. *differs significantly from the controls ($p < 0.05$); n = 6 animals/group.

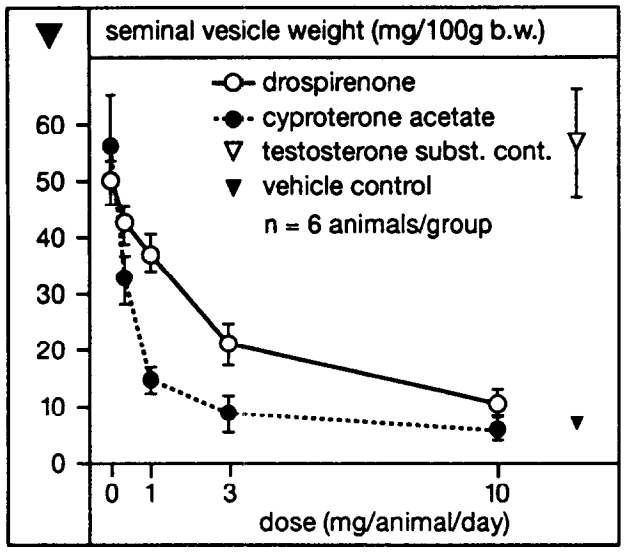
compounds are devoid of androgenic activity (data not shown).

Evaluation of Long-Term Antimineralocorticoid Activity of Drospirenone in Female Rats

A single dose of drospirenone has already been shown to have a potent natriuretic effect in rats.[14] Specifically, it was about eight times as powerful as spironolactone. The investigations reported here explored the persistence of the antimineralocorticoid effect on repeated application to ovariectomized rats.

Ovariectomized rats were treated for 21 days with various doses of drospirenone or spironolactone (0.1, 1, 10 mg/animal/day). Drospirenone was more potent than spironolactone in increasing the Na^+/K^+ excretion rate on the first day of treatment (Figure 4). However, as judged from the increased serum aldosterone levels at the end of the experiment (Figure 5), counterregulation was induced by both of the compounds. This may well explain the rapid regression of the antimineralocorticoid effect of spironolactone after the initial treatment phase and the smaller reduction found with drospirenone. However, a dose of 10 mg drospirenone/animal/day resulted in sustained stimulation of the Na^+/K^+ excretion ratio. Thus, drospirenone is to be regarded as a long-term antimineralocorticoid on repeated application of 10 mg/animal/day.

a)



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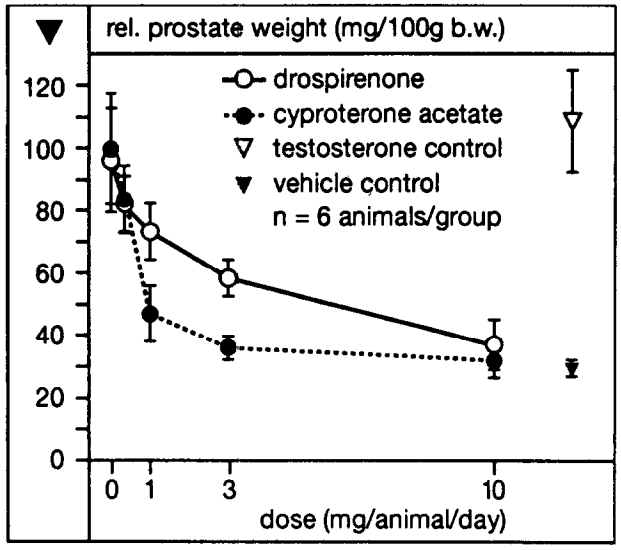

Figure 3. Antiandrogenic effect of drospirenone in castrated rats. Juvenile male rats were castrated. Beginning seven days later, they were substituted with testosterone propionate (0.1 mg/animal/day) and treated for 7 days with vehicle alone or different doses of drospirenone or cyproterone acetate as described under Methods. Relative organ weight was obtained by normalizing the wet weight to 100 g body weight. a) Reduction of seminal vesicle weight. b) Reduction of prostate weight.

Glucocorticoid and Antiglucocorticoid Activity

The adrenal gland is very sensitive to changing ACTH levels. Glucocorticoids, which inhibit ACTH secretion, lead to a progressive loss in organ weight. Adrenal weight is stimulated by treating the animal with antiglucocorticoids.

Gluco- and antiglucocorticoid activity was assessed by six weeks' application of 5 mg drospirenone/animal/day s.c. or p.o. to intact male rats. No change in adrenal weight was observed (see adrenal weight data in Table 3), which indicates that drospirenone is devoid of gluco- and antiglucocorticoid activity.

Another sensitive assay of gluco-/antiglucocorticoid activity is the regression of thymus weight in adrenalectomized juvenile rats. Application of drospirenone at a dose of 10 mg/day s.c. for four days did not show any significant suppression of thymus weight (Table 5), whereas thymus involution was stimulated considerably by dexamethasone at 0.01 mg/animal/day. This result confirms that drospirenone is devoid of glucocorticoid activity.

The antiglucocorticoid activity of drospirenone was also evaluated in comparison with mifepristone (RU 486) and CPA. Mifepristone caused a dose-dependent inhibition in dexamethasone-stimulated loss of thymus weight with an ID_{50} of about 10 mg/animal/day s.c. (Figure 6). Drospirenone produced 9.8% inhibition at a dose of 10 mg/animal/day and in this respect was comparable to CPA. The antiglucocorticoid activity of drospirenone can be seen to be negligible.

Evaluation of Estrogenic Activity

The estrogenic activity of drospirenone was assessed using the Allen-Doisy test. Uterine wet and dry

Table 4. Effect of drospirenone and cyproterone acetate on the urogenital sinus and external genital organs in male rat fetuses (feminization/virilization test s.c.)

			Urogenital Sinus					External Genital Organs				
			Length		Prostate Anlage (%)			Anogenital Distance		Presence of the Ventral Glandar Lamella (%)		
Substance	Dose (mg/animal/day s.c.)	n*	In mm (x ± SD)	Feminization vs. Controls	e†	ne‡	nep§	In mm (x ± SD)#	Feminization vs. Controls	e	ne	nep
Drospirenone	0.1	12	4.9 ± 0.3	11.4%	100			2.3 ± 0.3	14.9%	100		
	0.3	9	4.9 ± 0.2	9.3%	100			2.5 ± 0.3	4.4%	77.8	11.1	11.1
	1.0	12	4.9 ± 0.3	9.9%	100			2.5 ± 0.3	1.5%	83.3	16.7	
	3.0	13	4.3 ± 0.6	30.7%	92.3	7.7		2.0 ± 0.3	35.3%	7.7	84.6	7.7
	10.0	13	3.2 ± 0.8	72.4%	53.8	46.2		1.5 ± 0.4	67.6%		100	
Cyproterone	0.1	11	4.8 ± 0.3	13.7%	100			2.3 ± 0.2	16.5%	45.5	27.2	27.3
Acetate	0.3	12	5.0 ± 0.5	5.8%	100			2.4 ± 0.2	8.2%	25.0	66.7	8.3
	1.0	14	4.6 ± 0.3	20.7%	71.4	28.6		2.2 ± 0.3	19.8%	21.4	71.4	7.2
	3.0	12	4.0 ± 0.5	42.6%	66.7	25.0	8.3	1.8 ± 0.4	49.3%		100	
	10.0	16	2.7 ± 0.3	87.1%	31.3	62.5	6.2	1.3 ± 0.2	77.9%		100	
Vehicle Control	0.2 ml	18	5.2 ± 0.3		94.4		5.6	2.6 ± 0.4		83.3	11.1	5.6

n*: number of fetuses. e†: existent. nep‡: no evaluation possible. ne§: nonexistent. SD#: standard deviation. Underlined numbers indicate significant difference ($p < 0.05$) between treatment and control group.

weight, which is increased by estrogenic compounds in a dose-dependent manner, was also analysed. Drospirenone (10 mg/animal) produced no sign of estrogenic effects on the vaginal epithelium. A marginal stimulation of rat uterine wet but not dry weight was noted (Table 6). In contrast, estradiol induced a full vaginal reaction and a distinct increase in uterine wet and dry weight. These data indicate that drospirenone has no estrogenic activity.

Investigations into the Hormonal Interaction of Drospirenone and Ethinylestradiol

Combined or sequential treatment with estrogens and progestogens provides the basis of both oral contraception and hormone replacement therapy.

For this reason, it is important to investigate the interaction of drospirenone and estrogens, especially ethinylestradiol, in appropriate animal models.

MAINTENANCE OF PREGNANCY WITH A COMBINATION OF DROSPIRENONE AND ETHINYLESTRADIOL. Pregnancy is maintained in ovariectomized rats substituted with estrogen and progestogen.

Ovariectomized pregnant rats were substituted with drospirenone (3 mg/animal/day s.c.) and doses of ethinylestradiol (EE), varying between 0.01 and 3 μg/animal/day. In intact control animals, the maintenance rate was 93%. A comparable rate was obtained with a combination of 3 mg drospirenone and 0.1 μg EE/animal/day. As is the case with other estrogens, lower and higher EE doses led to decreased pregnancy maintenance rates (Table 7).

ALDOSTERONE ANTAGONISTIC EFFECT OF DROSPIRENONE IN COMBINATION WITH ETHINYLESTRADIOL (EE). The antimineralocorticoid action of drospirenone in combination with EE was evaluated in adrenalectomized, glucocorticoid-substituted male and female rats. The animals were treated with a single dose of EE and drospirenone. One hour later, a 15-hr infusion of a NaCl/ glucose solution was started with or without the addition of aldosterone (1 μg/kg/h). Urine fractions were collected over the entire time range and analysed for Na^+ and K^+ content. Borderline influence on aldosterone-driven Na^+-excretion was observed between 0.5 (females) and 1 mg (males) drospirenone/rat. In neither sex was EE (1 and 10 μg/animal/day) found to have any effect on the test parameters (data not shown). Thus, EE does not interfere with the antimineralocorticoid activity of drospirenone.

Discussion

The experimental data reveal that drospirenone is a compound with progestogenic (rabbit, rat, monkey), antiandrogenic (rat, monkey) and aldosterone antagonistic (rat) activity.

The progestogenic activity of drospirenone has

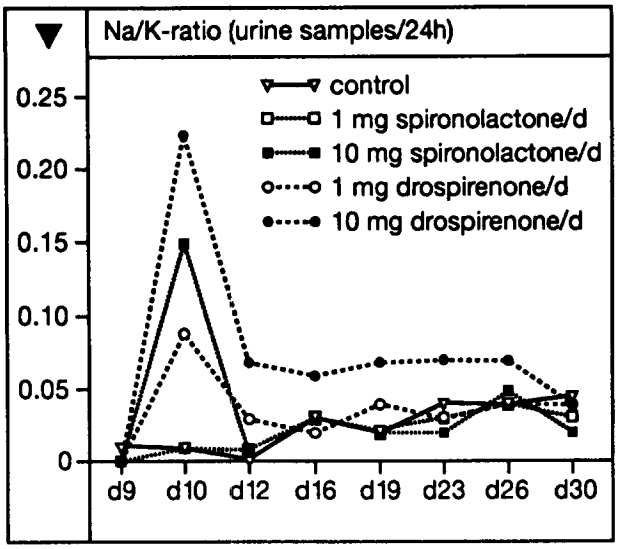

Figure 4. Effect of repeated application of drospirenone and spironolactone on the urinary Na^+/K^+ excretion ratio in ovariectomized rats. Female rats were ovariectomized on day 1. The animals were treated daily with drospirenone or spironolactone from day 9 to 29. Urine was collected for 24 hrs on days 9, 10, 12, 16, 19, 23, 26, and 30. Sodium and potassium levels were determined by flame photometry; n = 6 animals/group except for vehicle control (n = 12 animals/group).

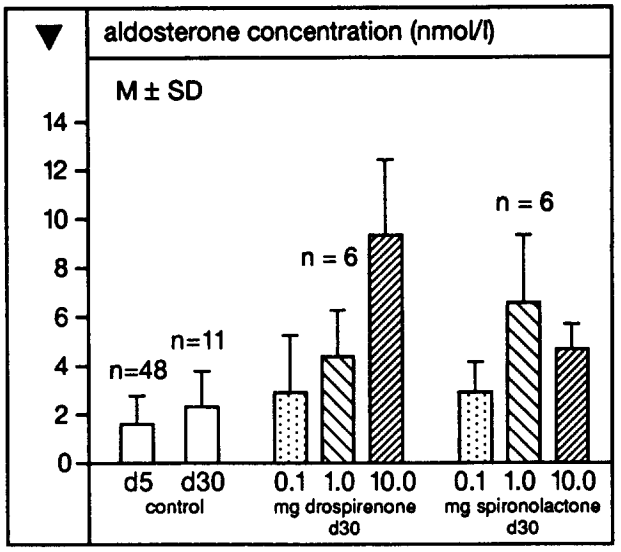

Figure 5. Serum aldosterone concentration in ovariectomized rats before (day 5) and after (day 30) a 21 days' treatment (s.c.) with drospirenone or spironolactone. Animals were treated from day 9 to day 29; M ± SD: mean ± standard deviation.

Table 5. Suppression of thymus weight induced by drospirenone and mifepristone in comparison with dexamethasone after four days' treatment of adrenalectomized juvenile male rats

	Treatment (mg/animal)	Relative Thymus Weight (mg/100 g Body Weight)	% Suppression as Compared With Control
Control	Vehicle	358 ± 56	—
Drospirenone	10	350 ± 61	2.2
Mifepristone	10	306 ± 24	14.4*
Dexamethasone	0.01	81 ± 13	77.4*

n = 7 to 10 animals/group; * indicates a significant difference vs. control.

been evaluated in a variety of animal models. The results obtained in the rat (maintenance of pregnancy) and in the rabbit (endometrial transformation) suggest a relative progestational potency of the same order as that of norethisterone acetate. Drospirenone also showed antigonadotropic activity; its antiovulatory potency in the rat was found to be comparable to that of norethisterone acetate (for comparison see Ref. 20).

Drospirenone shows no androgenic activity *in vitro* as has been demonstrated by a lack of stimulation of androgen receptor driven gene transcription (Fuhrmann et al., unpublished data). Similar results were obtained *in vivo*: treatment of castrated male rats with drospirenone had no influence on the weight of the accessory sex organs (seminal vesicles, prostate). Moreover, the substance had no recognizable virilization effect on the process of sexual differentiation of female rat fetuses. These results demonstrate that drospirenone is devoid of any androgenic activity.

The antiandrogenic effect of drospirenone has been investigated in a transactivation assay *in vitro* by means of stable transfection of a cell line with the androgen receptor and a reporter gene.[12] Inhibition of the androgen receptor-induced reporter gene activity was dose-dependent (Fuhrmann et al., unpublished data). The IC_{50} of drospirenone was somewhat higher than that of cyproterone acetate. *In vivo*, antiandrogenic activity was demonstrated by inhibition of androgen-stimulated growth of the accessory sexual organs (seminal vesicles, prostate) in testosterone-substituted, castrated male rats. The potency of drospirenone *in vivo* was found to be about a third that of cyproterone acetate on s.c. application. The results obtained *in vivo* as well as data concerning inhibition of androgen-stimulated gene transcription *in vitro* (Fuhrmann et al., unpublished) imply that the antiandrogenic activity of drospirenone is based on a functional blockade of the androgen receptor.

Drospirenone also showed antiandrogenic activity upon oral application of a daily dose of 3 to 10 mg/animal. This contrasts with the results obtained in the maintenance of pregnancy test, where the dose

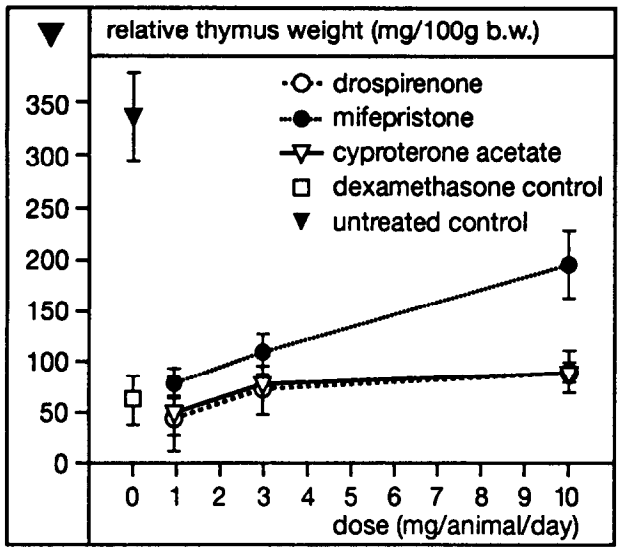

Figure 6. Inhibition of dexamethasone-induced thymus weight involution. Adrenalectomized juvenile male rats were substituted with dexamethasone and treated for four days with drospirenone, cyproterone acetate or mifepristone as described under Methods. Thymus wet weight was determined and normalized to 100 g body weight.; n = 7 animals/group except for dexamethasone treatment (n = 10).

Table 6. Uterine weight and vaginal reaction of rats after treatment with drospirenone and estradiol

Treatment (s.c.)	Dose	Relative Uterus Weight		Vaginal Reaction
		Wet	Dry	
Control	—	49.3 ± 4.3	10.0 ± 0.9	no
Drospirenone	10 mg	62.6 ± 6.7	10.7 ± 1.2	no
Estradiol	1 μg	115 ± 6.3	17 ± 1.8	yes

Animals were treated with a single dose of drospirenone or estradiol. Vaginal smears and uterine weight were investigated as described under Methods. n = 6 animals/group.

had to be split into six applications in order to obtain maximal activity. These divergent results are probably due to the relatively short half-life (but high bioavailability; data not shown) of drospirenone in rats. Whereas maintenance of pregnancy apparently requires continuously high blood levels, the occurrence of antiandrogenic effects is less affected by the pharmacokinetics of drospirenone.

Drospirenone induced feminization in male fetuses following treatment of pregnant rats at a dose of 3 mg/day during the vulnerable period of sexual differentiation. This is a property it has in common with all potent antiandrogens.[10,13] Consistent with the antiandrogenic potencies of the two compounds, drospirenone was about a third to a tenth as active in this

Table 7. Maintenance of pregnancy with a combination of drospirenone and ethinylestradiol

Group			Maintenance of Pregnancy (%)
Intact Controls			92.9
Ovariectomized Controls			0
Drospirenone (mg/animal/day)		**Ethinylestradiol (μg/animal/day)**	
3	+	0.01	50.0
3	+	0.03	75.0
3	+	0.1	91.7
3	+	0.3	80.0
3	+	1	0
3	+	3	0

Ovariectomized pregnant rats were substituted with 3 mg/animal/day of drospirenone and diverse doses of ethinylestradiol. The pregnancy maintenance rate was determined as described under Methods. n = 11 animals/group.

respect as cyproterone acetate. Based on these data and taking into consideration the clinical experience with pharmacological preparations containing CPA, feminization of human fetuses at ovulation inhibitory doses of drospirenone can be excluded.

Drospirenone is a potent antimineralocorticoid *in vitro* and *in vivo*. This is an outstanding characteristic and has not been described for any other synthetic progestogen at therapeutically relevant doses. The natural hormone progesterone, however, exhibits this activity (Ref. 3–7; Fuhrmann et al., unpublished).

The antimineralocorticoid properties of drospirenone and progesterone have been demonstrated in the rat distal colon in vitro where both compounds inhibited aldosterone-stimulated transepithelial sodium transport.[15] As has been reported earlier,[14] short-term studies with adrenalectomized, aldosterone-substituted rats indicate that drospirenone stimulates sodium and water excretion in a dose-dependent manner. It has now been shown that the compound also exhibits long-term antimineralocorticoid activity in rats upon treatment for 21 days with 10 mg/animal/day. Under these conditions, the compound stimulated the sodium/potassium excretion ratio over the entire treatment period while spironolactone became ineffective after the first or second application. This is probably due to the induction of counter-regulation: the increased serum aldosterone levels observed under the treatment apparently completely overcame the natriuretic effect of spironolactone but not that of drospirenone.

Additional endocrine pharmacological investigations showed that drospirenone is devoid of estrogenic, glucocorticoid and antiglucocorticoid activity as can be seen from the absence of an influence on

vaginal epithelial cornification in rats, adrenal weight changes in rats, and thymus regression in adrenalectomized, glucocorticoid-substituted rats, respectively.

The interaction between ethinylestradiol and drospirenone was investigated in relevant animal models. Drospirenone led to maintenance of pregnancy in rats after priming with either estrone or ethinylestradiol. Data on Na^+ excretion in rats showed that, even at high doses, ethinylestradiol does not interfere with the antimineralocorticoid activity of drospirenone. The absence of drug interference between EE and drospirenone in the animal models suggests drospirenone and EE would be a clinically effective combination, for example in OCs.

Drospirenone is expected to act as a progestogen with aldosterone antagonistic and antiandrogenic properties in women. It has already been shown that drospirenone exhibits strong central and peripheral progestational activity in humans[17] which renders the compound suitable for a variety of applications including oral contraception and hormone replacement therapy. Drospirenone has also been shown to exhibit antimineralocorticoid activity on oral application to women.[17]

Drospirenone's lack of androgenicity and its antiandrogenic activity suggest the compound may have positive effects in women suffering from symptoms of androgenization such as acne and seborrhea. Its antimineralocorticoid activity is expected to result in reduced weight gain and in an amelioration of other side effects possibly related to estrogen-induced water retention, such as breast tension, nausea and headache, quite common complaints among women taking estrogens or OCs.

The absence of aldosterone antagonistic activity in the synthetic progestogens available today may also be connected with the discrete increase in blood pressure[21,22] which is observed in a number of OC users. A beneficial effect of drospirenone on blood pressure development is to be inferred from the results of a study involving 4 weeks' treatment of *spontaneously hypertensive rats* (SHR) with equipotent progestogen doses of drospirenone, progesterone, levonorgestrel and cyproterone acetate. Whilst the last two compounds caused an increase, with drospirenone a slight reduction of blood pressure was registered.[23]

In conclusion, drospirenone is a novel progestogen with antimineralocorticoid and antiandrogenic characteristics. It is devoid of androgenic, estrogenic, gluco- and antiglucocorticoid properties. Drospirenone thus exhibits an innovative pharmacodynamic profile, which is more closely related to that of the natural hormone progesterone than is that of any other synthetic progestogen in use today. With such a profile, drospirenone is expected to provide a number of additional health benefits both in OC users and hormone replacement therapy recipients.

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