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ANTI-PROSTATIC ACTIVITY OF BIFLURANOL, A FLUORINATED BIBENZYL

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1 Endocrine and anti-fertility studies were carried out on a fluorinated bibenzyl, bifluranol, in rats and mice.

2 A potent anti-prostatic activity of bifluranol was shown to be comparable to diethylstilboestrol (DES). In contrast, its oestrogenic potency by the oral route was about eight times less than that of DES.

3 In comparative short and long-term anti-androgenic and fertility studies in rats and in studies on sexual potency and reproductive performance in male mice, bifluranol given orally was shown to produce fully reversible suppression of accessory sexual structures without impairment of spermatogenesis and fertility. In contrast, DES administered in the same dose reduced spermatogenesis as well as accessory sexual glands.

4 Bifluranol lowered serum luteinising hormone (LH) levels without affecting follicle stimulating hormone (FSH). Under similar conditions DES reduces both LH and FSH levels. Since bifluranol does not antagonize androgen-induced stimulation of the prostate in castrated rats, its anti-prostatic effect is interpreted as a negative, hormonostatic feedback activity, mediated through a selective inhibition of LH secretion.

Introduction

Early attempts to separate anti-fertility from oestrogenic activity in non-steroidal compounds have led to the synthesis of a number of stilbene and bibenzyl derivatives related to diethylstilboestrol (DES) and hexoestrol. They have been found to combine a high anti-fertility effect with low oestrogenic activity (Emmens, 1965; Collins & Hobbs, 1967; Emmens, Collins, Hobbs, Miller & Owen, 1968). More recently bifluranol (Figure 1) has been synthesized and examined in an attempt to separate anti-prostatic from oestrogenic actions and the study of its properties is presented in this paper. Pharmacokinetics, LD_{50}, repeat dose toxicity, haematological and blood coagulation studies and histopathological evaluation of bifluranol will be described elsewhere.

Methods

Albino mice and Biorex Wistar rats of a single strain bred in these laboratories were used for the endocrine and anti-fertility tests. The animals were kept at about 60% relative humidity and $\pm 22°C$ with free access to Dixon's FFG (M) diet and water under a cycle of 14 h light alternating with 10 h darkness. All compounds were dissolved in redistilled isopropyl myristate (IPM) and were administered subcutaneously or orally by a stainless steel stomach tube in a volume of 0.05 or 0.1 ml. Some tests were carried out in parallel with reference preparations: oestradiol-17β, DES or methyltestosterone.

Androgenic activity in rats

This was assessed indirectly by evidence of changes of accessory sexual glands in male rats used for the test on gonadotrophin release.

Anti-androgenic activity in castrated rats

Groups of 10 immature rats (50 g) were bilaterally orchidectomized 14 days before the test. Daily doses of bifluranol were given subcutaneously for 7 days. Methyltestosterone (MT) was injected concomitantly but at a separate site. Twenty four hours after the last injection, the animals were killed and the weights of body, ventral prostate and seminal vesicles were recorded.

Suppression of gonadotrophin release in rats

Groups of 8 male rats (approx. 200 g) were dosed orally for ten consecutive days with bifluranol 0.2 mg/kg and 0.8 mg/kg or DES 0.2 mg/kg, one group

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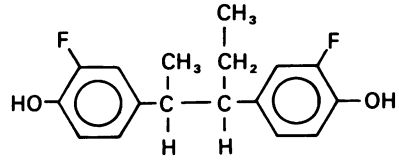
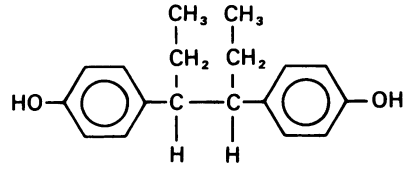
Bifluranol



Hexoestrol



Diethylstilboestrol

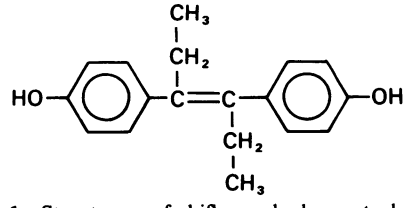


Figure 1 Structures of bifluranol, hexoestrol and diethylstilboestrol.

serving as the vehicle control. Twenty-four hours after the last intubation the animals were weighed and blood samples collected after decapitation. Individual serum samples were prepared and immediately deep-frozen (−18°C) for luteinizing hormone (LH) and follicle stimulating hormone (FSH) assays. The concentrations of LH and FSH were measured in triplicate by the double anti-body radioimmunoassay as described by Naftolin & Corker (1971), using LH standard LER- C_2-1056, provided by Dr L.E. Reichart, and anti-body to LH No. 15, provided by Dr G.D. Niswender. FSH was assayed similarly using standard and anti-body provided by the Hormone-Distribution Officer, National Institutes of Health Bethesda, U.S.A.

At autopsy the testes, ventral prostate gland, seminal vesicles, pituitary gland, adrenal glands, thymus and levator ani muscle were dissected out and weighed while fresh, the levator ani muscle being dried at 60°C for 24 h before weighing.

Comparative oral long-term anti-androgenic and anti-spermatogenic activity in rats

The studies were made in comparison with DES in an attempt to estimate first, the anti-androgenic dose that suppresses the accessory sexual glands without affecting spermatogenesis and secondly the anti-spermatogenic dose which leads to a reversible sterility. Adverse reactions were also recorded and compared. The studies were divided into two parts: (A) treatment and (B) recovery, four groups including vehicle control group of 36 rats (200 g) being used. In the treatment study (A), each rat was dosed daily by stomach tube for 42 consecutive days and six rats from each group were killed at 10, 21 and 42 days. In the recovery study (B), after 6 weeks treatment, a further six rats from each group were killed at 8, 12 and 15 weeks. In addition six rats of each group to be killed at 15 weeks were investigated for fertility by mating each week with three females to a male for five consecutive days. Day 1 of pregnancy was recorded by daily sperm identification in the vaginal smears and the pregnancy confirmed on Day 12 by the presence of a number of embryos and implantation sites. The pregnant animals were replaced by fresh females at the beginning of each week.

Individual body weights were recorded once a week throughout. From these individual figures the group mean body weight per rat was calculated. The food consumption and water intake were measured daily. The quantity of food consumed by the rats in each cage was recorded and mean weekly intake, g/kg body weight calculated. Water intake was read on external scales marked on the water bottles. At post-mortem examination the following organs were dissected out and weighed while fresh: testes, ventral prostate gland, seminal vesicles, pituitary gland, adrenal glands, kidneys and liver. In addition, the levator ani muscle of each rat was dissected after 6 weeks of treatment and at 9 weeks of the recovery period and dried at 60°C for 24 h before weighing.

Sexual behaviour, potency and fertility in male mice

Groups of 10 and 20 male mice (30 to 35 g) were placed in individual cages and on the day before the routine mating procedure, oral treatment was started with various doses (0.14 mg to 4.56 mg/kg daily) of bifluranol and continued for 20 days. Two groups served as a vehicle control and untreated control, respectively. On the day of mating, four females (28 g) were placed with a male, and their behaviour was carefully observed. The mice were examined daily for the presence of the vaginal copulatory plugs. Pregnant animals were separated and allowed to proceed to term. On the day of parturition the gestation period and the size of litters were recorded and each newborn animal was weighed and examined for gross external anomalies. The male mice were killed on Day 21 of the experiment and the body and organ weights (testes, ventral prostate gland and seminal vesicles) recorded.

Oestrogenic activity

This was assessed by standard techniques: the uterine weight response in immature mice (Rubin's test) and the vaginal cornification response (Allen-Doisy test) in ovariectomized mice. Immature mice (8 to 10 g), in groups of 20, were injected subcutaneously for 3 days at various doses, including 2.5 μg and 5.0 μg/kg. The uterine/body weight ratio was calculated by dividing the uterine weight in mg by the body weight in g, multiplied by 100. Ovariectomized mice (25 g), in groups of 20, were injected subcutaneously or intubated orally at various doses as a single administration. The median effective dose (MED) was calculated from all positive vaginal smears, examined at each dose level, which contained only cornified cells or occasionally nucleated cells but no leucocytes.

Results

Androgenic activity in rats

The results shown in Table 2 show that bifluranol, like DES, produced no stimulation but only a significant, dose-related reduction in the weight of both prostate and seminal vesicles.

Anti-androgenic activity in castrated rats

Bifluranol did not antagonize the MT-induced stimulation of the prostate gland at doses ranging from 0.5 mg to 8 mg/kg s.c. (Table 1). However, at the high dose level it potentiated the MT-induced growth of the seminal vesicles, presumably reflecting a known phenomenon that oestrogens variably antagonize the effects of androgens on the secondary sexual structures when both are administered to castrated rats (Briggs & Brotherton, 1970). The retardation of body growth seems unlikely to be related to anti-androgenic properties of bifluranol given by both subcutaneous and oral routes. The results in Figure 2 suggest a direct effect.

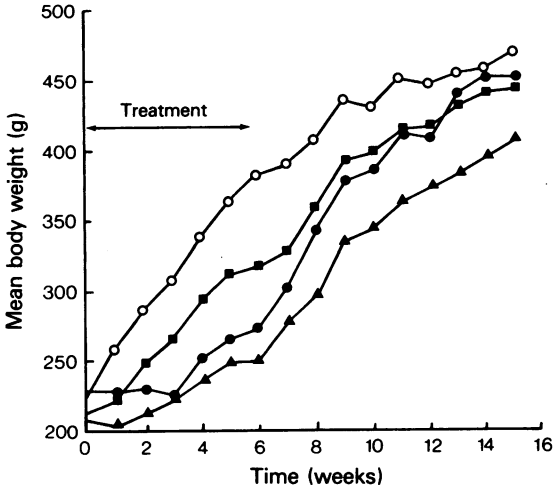


Figure 2 Mean body weight of male rats treated by stomach tube with diethylstilboestrol 0.2 mg/kg (●), bifluranol 0.2 mg/kg (■) and 0.8 mg/kg (▲) for 42 consecutive days. Vehicle controls (○) received isopropyl myristate 0.1 ml/day. The values are means of 6 observations.

Suppression of gonadotrophin release in rats

No change was observed in testes weight at the lower dose of bifluranol but in the higher dose group and the DES group a significant reduction in testes weight was noted (Table 2). The weights of the prostate gland, seminal vesicles and levator ani muscle were considerably reduced in all treated groups. A significant increase in pituitary weights was noted in rats on DES treatment but no such effect was apparent with

Table 1 Anti-androgenic activity of bifluranol in castrated rats as reflected in mean body and organ weights

Treatment	*Daily dose* (mg/kg)	*Final body (weight)* (g)	*Ventral prostate* (mg)	*Seminal vesicles* (mg)
Vehicle control	0	162 ± 6.4	25 ± 2.0	14 ± 0.9
Methyltestosterone (MT)	2	160 ± 6.4	57 ± 2.9†	57 ± 3.0†
MT + bifluranol	2 + 4	133 ± 3.4**	55 ± 4.2	63 ± 3.1
MT + bifluranol	2 + 8	131 ± 2.8***	54 ± 4.1	68 ± 2.8*

Daily doses were given subcutaneously for 7 days in 0.1 ml volume.
Values represent the mean ± s.e. mean of 10 observations.
Significance (*t* test); †$P < 0.001$ from control; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ from methyltestosterone alone.

Table 2 Comparative effects of bifluranol and diethylstilboestrol on serum luteinising hormone (LH) and follicle stimulating hormone (FSH), body weights and organ weights in rats

Treatment	*Dose* (mg/kg)	*Body weight gain* (g)	*Serum LH*	(ng/ml) *FSH*	*Testes* (g)	*Ventral prostate* (mg)	*Seminal vesicles* (mg)	*Levator ani* (mg)	*Pituitary* (mg)	*Adrenals* (mg)	*Thymus* (mg)
Vehicle control	0	62 ±1.9	1.10 ±0.24	282.5 ±40.3	2.7 ±0.1	268 ±23.6	282 ±18.5	33.5 ±2.6	8.1 ±0.2	44 ±1.5	497 ±20
Bifluranol	0.2	21*** ±2.7	0.50* ±0.11	277.7^{NS} ±22.9	2.6^{NS} ±0.1	90*** ±5.0	93*** ±4.4	14*** ±0.8	8.6^{NS} ±0.2	60*** ±2.7	390** ±21
Bifluranol	0.8	17.4*** ±4.6	0.49** ±0.17	246.5^{NS} ±19.0	2.3* ±0.1	71*** ±8.0	82*** ±3.5	12*** ±0.7	8.5^{NS} ±0.3	66*** ±4.9	377** ±29
Diethylstilboestrol	0.2	8.1*** ±4.9	0.06*** ±0.04	172.2* ±19.4	2.3* ±0.1	105*** ±7.1	107*** ±5.3	18*** ±1.7	9.0* ±0.4	66*** ±4.7	372** ±23

Daily doses were given in 0.1 ml volume by stomach tube for ten days.
The results represent the means ± s.e. mean of 8 observations.
Significance from control (*t* test): $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. NS not significant.

bifluranol. As would be expected with oestrogenic compounds, the weight of the adrenal gland was moderately increased while that of the thymus decreased in all treated groups. Comparative measurement of serum LH and FSH show that bifluranol at both 0.2 mg/kg and 0.8 mg/kg reduced selectively LH levels without affecting FSH. In contrast, DES at 0.2 mg/kg exerted a negative feed-back effect on both LH and FSH gonadotrophins as expected.

The effects of both bifluranol and DES on body weights were similar and reflected an apparent retardation of the growth rate during a short period of treatment.

Comparative oral long-term anti-androgenic and anti-spermatogenic effects in rats

The weight of testes in the bifluranol group receiving 0.2 mg/kg was only moderately affected and increased steadily at a slow rate over 6 weeks of treatment, reaching the weight of the vehicle control group at 2 weeks recovery. However, both the DES group (0.2 mg/kg) and the high bifluranol group (0.8 mg/kg) showed marked reduction in the weight of testes over 6 weeks of treatment. After 2 weeks recovery, slight improvement was observed and after 6 weeks the weights of testes between individual animals within any group or between the treated and vehicle control groups were indistinguishable. The testicular reproductive function was reflected by a return of potency and fertility in rats intubated with 0.2 mg/kg bifluranol over the treatment period but not in those intubated with 0.2 mg/kg DES and 0.8 mg/kg bifluranol. During the recovery period testicular function returned gradually to normal in all groups. By contrast, there was little difference in weight and size of prostate glands and seminal vesicles during the 6 weeks of treatment, these glands being almost equally reduced in all treated groups. On cessation of treatment, moderate increases in the glands and seminal vesicle were observed at 2 to 6 weeks recovery with full recovery after 9 weeks. The weights of levator ani muscles were also reduced in all treated groups with full recovery after 9 weeks. There was no apparent increase in the weight of the pituitary gland although enlargement of the adrenal gland was noted in all test animals during treatment. In all treated groups, kidney and liver weights were reduced in proportion to body weight changes, but returned fully to those of controls during the recovery period. During the 6 week treatment the lower bifluranol group (0.2 mg/kg) remained fertile while the DES group (0.2 mg/kg) and the high bifluranol group (0.8 mg/kg) became infertile and probably impotent as no sperm could be identified in the vaginal smears. The DES and high bifluranol groups regained the levels of fertility shown by the low bifluranol group at 6 weeks in about 3 weeks

after cessation of treatment. During treatment, moderate alopecia occurred in rats receiving both bifluranol and DES. The hair-coat returned to normal within a few weeks of the recovery period. All treated animals showed an apparent reduction of the body growth, DES group for 3 weeks, the high bifluranol group for 2 weeks and the low bifluranol group for 1 week. The growth rate assumed a normal pattern during the recovery period, (Figure 2). In the first 3 weeks of the treatment, food consumption was moderately reduced in the treated groups. The limited reduction in food intake is unlikely to be wholely responsible for the retardation of body growth as a centrally mediated systemic effect could also be involved.

Effect on the sexual behaviour, potency and fertility in male mice.

The only apparent effect was the reduction in size and weights of accessory sexual glands in all treated groups under the conditions of these experiments. At the low oral daily doses (0.14 mg and 0.57 mg/kg) bifluranol produced moderate reduction in weights of the prostate gland and seminal vesicles but with no accompanying suppression of spermatogenic activity or reproductive performance, as 86 and 95% females littered, respectively. Higher doses (1.14 mg, 2.28 mg and 4.56 mg/kg) produced a more marked reduction of prostate gland and seminal vesicles weights and possibly some anti-spermatogenic effect, but the size and weight of testes were still unaffected. The reproductive performance was only slightly reduced, the pregnancy rate being 87, 72 and 57%, respectively. All females that littered gave birth to normal offspring.

Oestrogenic activity

The uterine/body weight ratio of 297 for bifluranol at 5 μg/kg (s.c.) reflects a rather weak uterotrophic potency in comparison with the oestradiol ratio of 404 and the DES ratio of 560, at equal dose levels. In the vaginal cornification test, the median effective dose (MED) of 18 μg/kg signifies a moderate (s.c.) oestrogenic activity of bifluranol in comparison with the MED of 13 μg/kg for both oestradiol and DES. However, by the oral route, bifluranol (MED, 108 μg/kg) is about 8 times less potent than DES (MED, 13 μg/kg).

Discussion

Bifluranol, a weak oestrogen, was found to act as a potent anti-prostatic agent through a selective inhibition of pituitary LH secretion without affecting FSH secretion, as reflected by atrophy of accessory sexual structures without changes in the function of testes or reproductive performance. In contrast, DES at equivalent dose levels reduces both LH and FSH secretion and exerts a negative feed-back effect on both gonadotrophins and in this way affects spermatogenesis, as well as accessory sexual structures.

These results seem to indicate that bifluranol, in contrast to DES, selectively reduces LH secretion and exhibits much greater anti-prostatic activity than its negligible oral oestrogenicity could explain.

Bifluranol could be acting at the hypothalamic level inhibiting release of a releasing hormone which only affects secretion of LH. According to Schally, Kastin & Arimura (1972) this is unlikely as gonadotrophin-releasing hormone stimulates the release of both LH and FSH from the hypophysis. An alternative and perhaps more likely hypothesis is that bifluranol is active at the pituitary level, inhibiting selectively LH secretion.

The validation of this hypothesis of bifluranol action would need further information on the mode of binding to the specific receptor sites, e.g. on the hypothalamus and hypophysis. In addition, the studies should be carried out on the role of pituitary prolactin which may interfere with gonadotrophin release and affect gonadal functions individually or by synergistic action with bifluranol.

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