AN OVERVIEW OF ANIMAL TOXICOLOGY STUDIES WITH BICALUTAMIDE (ICI 176,334)

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ABSTRACT — The toxicological profile of bicalutamide in animals following acute and chronic dosing is closely associated with the drug's non-steroidal anti-androgenic pharmacological activity. Bicalutamide produces typical effects of an anti-androgen, including atrophy of the prostate, testis and seminal vesicles and Leydig cell hyperplasia resulting from inhibition of pituitary feedback by testosterone. Subsequent benign Leydig cell tumors were seen in rats, but Leydig cell hyperplasia has not been observed in patients.

Bicalutamide causes liver enlargement and is a mixed function oxidase inducer in rodents and dogs, but not man. These effects lead to thyroid hypertrophy and adenoma in the rat and hepatocellular carcinoma in the male mouse. *In vitro* and *in vivo* genotoxicity studies have all given negative results.

Bicalutamide also caused a reversible shortening of the electrocardiographic P-R interval in the dog without any associated pathology. This change was not detected in ECG monitoring during clinical trials.

In conclusion, bicalutamide produced a range of pharmacological effects, as well as liver enlargement with enzyme induction and dog ECG changes in preclinical toxicity studies in rodents and dogs. Only the pharmacological changes were found to be relevant to human usage.

KEY WORDS : Bicalutamide, ICI 176,334, Anti-androgen, Toxicity

INTRODUCTION

Bicalutamide is a potent, non-steroidal anti-androgen which induces little or no stimulation of the hypothalamic-pituitary axis in several animal models (Furr, 1989). It binds to androgen receptors without activating androgen-responsive gene expression, and thus inhibits the androgenic stimulation of androgen-dependent tissues. As regression of prostatic tumors results from this inhibition, the drug has the potential for therapeutic use in androgen-dependent conditions such as prostate cancer. The battery of experimental pharmacological studies conducted in support of its use in prostate cancer revealed potent inhibitory effects on growth of human

prostate tumor cell lines (Veldscholte *et al.*, 1992a; Veldscholte *et al.*, 1992b) and androgen-responsive rat Dunning tumors (Furr, 1989).

The chemical structure of bicalutamide is shown in Fig. 1. The molecule has one asymmetric center, and pharmacological activity is

Fig. 1. Chemical structure of bicalutamide (*: the position of the chiral center).

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restricted to the (R)-enantiomer. The compound used for clinical trials and most of the safety evaluation testing was a racemic mixture. Bicalutamide showed good oral bioavailability in all animal species used in toxicity studies. Toxicokinetic studies demonstrated that systemic exposure increased with dose in a non-linear manner (Cockshott *et al.*, 1991).

Table 1 summarizes the preclinical *in vivo* study details, including animal species, study duration and dose levels. Repeated-dose studies included six- and twelve-month studies in the rat and the dog, and two-year oncogenicity studies in the mouse and the rat. Additional *in vitro* studies of genotoxicity consisted of a battery of Ames tests, as well as human lymphocyte chro-

Table 1. Toxicological studies on bicalutamide.

Study	Species/strain	Route/duration/ type of study	Dose(mg/kg/day)	Number of animals group (sex)
Single-dose toxicity studies	mouse	oral	2000	5 (M & F)
	Alpk:APfCD-1	intraperitoneal	2000	5 (M & F)
	rat	oral	2000	5 (M & F)
	Alpk:APfSD (Wistar)	intraperitoneal	2000	5 (M & F)
	dog beagle	oral	500, 1000, 2000	2 (M)
Repeated-dose toxicity studies	rat	oral - 6 months	0, 10, 50, 250	20(M & F)
	Alpk:APfSD(Wistar)	oral - 12 months	0, 5, 15, 75	20-21(M& F)
	dog	oral - 6 months	0, 2.5, 10, 100	5 (M & F)
	beagle	oral - 12 months	0, 1, 2.5, 50	5 (M & F)
Reproduction studies	rat	oral - Segment I(♂)	0, 1, 10, 250	25 (M)
	Alpk:APfSD (Wistar)	oral - Segment II	0, 1, 10, 50, 250 ^{#1}	20-22 (F)
			0, 0.1, 1, 10, 250 ^{#2}	12-13 (F)
		oral - Segment III	0, 0.1, 1, 10, 250	20-22 (F)
	rabbit dutch belted	oral - Segment II	0, 10, 50, 200	18-20 (F)
Mutagenicity studies	rat	oral	0, 2000	5 (M & F)
	Alpk:APfSD(Wistar)	cytogenetics test		
	mouse CCBF ₁	intraperitoneal micronucleus test	0, 2000	5 (M & F)
Oncogenicity studies	mouse	oral (in diet)	0, 5, 15, 75	control group:100
	C57BL/10JfCD-1	2 years		treated group: 50 (M & F)
	rat	oral (in diet)	0, 5, 15, 75	control group:
	Alpk:APfSD (Wistar)	2 years		102-104 treated group: 51-52 (M & F)

#1: Observation of the fetuses at term of pregnancy.

#2: Observation of the offspring.

mosomal aberration, yeast gene mutation and gene conversion, and mammalian CHO cell mutation assays.

The single oral lethal dose in the mouse, rat and dog is in excess of 2000 mg/kg, and there was no evidence of acute toxicity in any of the three species at this high dose level. The findings in the repeated dose studies can be categorized into three types, i.e., (1) pharmacological effects, (2) consequences of enzyme induction, and (3) other toxicities, and are discussed below under these headings.

REVIEW OF SALIENT FINDINGS

Pharmacological effects

Pharmacological effects were seen in the male reproductive tract, the female reproductive

tract and the adrenal glands.

1. Male reproductive tract

The characteristic effects of anti-androgens were observed as atrophic changes in the prostate gland, seminal vesicles and testes. Table 2 gives the incidence of atrophic changes in these three organs. The pituitary-gonadal axis was also affected by anti-androgenic action, although poor penetration of bicalutamide into the central nervous system prevented the effects of hypothalamic androgen receptor blockade, such as the marked elevations in LH, associated with gonadectomy (Freeman *et al.*, 1989). Action at pituitary gonadotroph androgen receptors, causing some increase in LH secretion, accounted for the Leydig cell hyperplasia observed in both the rat and the dog. After six months' dosing, pituitary gonadotroph hypertro-

Table 2. Incidence of atrophic changes in prostate gland, seminal vesicles and testes in the 12-month rat and dog studies.

Findings	Rat (dose in mg/kg/day)				Dog (dose in mg/kg/day)			
	0	5	15	75	0	1	2.5	50
Body weight at week 52 ^{#1}	708	726	741	686	20.8	19.8	19.8	19.2*
(Mean $\pm$ SD)	± 49	± 72	± 86	± 55	± 2.6	± 2.9	± 2.9	± 2.6
	(20)	(19)	(20)	(20)	(5)	(5)	(5)	(5)
<u>Prostate</u> ^{#2}								
glandular atrophy								
- minimal/mild	0/21(0)	8/21(38)	4/21(19)	4/21(19)	0/5(0)	0/5(0)	0/5(0)	0/5(0)
- moderate	0/21(0)	8/21(38)	15/21(71)	8/21(38)	0/5(0)	0/5(0)	0/5(0)	0/5(0)
- severe	0/21(0)	0/21(0)	1/21(5)	8/21(38)	0/5(0)	5/5(100)	5/5(100)	5/5(100)
<u>Seminal vesicles</u> ^{#2}								
atrophy								
- minimal/mild	0/21(0)	14/21(67)	6/21(29)	3/21(14)				
- moderate	0/21(0)	2/21(10)	15/21(71)	16/21(76)				
<u>Testes</u> ^{#2}								
seminiferous tubule atrophy								
- minimal/mild	0/21(0)	1/21(5)	5/21(24)	6/21(29)	1/5(20)	1/5(20)	1/5(20)	2/5(40)
- moderate	1/21(5)	1/21(5)	8/21(38)	8/21(38)	0/5(0)	0/5(0)	0/5(0)	0/5(0)
- severe	2/21(10)	0/21(0)	1/21(5)	7/21(33)	0/5(0)	0/5(0)	0/5(0)	0/5(0)
Leydig cell hyperplasia	0/21(0)	7/21(33)	15/21(71)	19/21(90)	0/5(0)	4/5(80)	5/5(100)	5/5(100)

#1: Weight in grams for rats, kilograms for dogs. The number in parenthesis shows the number of animals per group.

#2: Number of animals with each finding / number of animals examined, (): %.

* : $P < 0.05$ compared with the control group (ANOVA followed by Student's t-test)

phy and vacuolation (so-called 'castration cells') were observed in dosed male rats. The magnitude of these effects was greater than the minor hormonal changes recorded in pharmacological studies, suggesting a progressive change on chronic dosing.

In the two-year rat oncogenicity study, there was the expected increase in incidence of both Leydig cell hyperplasia and neoplasia extending to all doses employed (the incidences of benign Leydig cell tumor in the 0, 5, 15 and 75 mg/kg/day groups were 2.9, 37.3, 62.7 and

Table 3. Incidence of findings in the ovary and uterus of rats in repeated dosing studies.

Findings	6-month treatment. (dose in mg/kg/day)				12-month treatment. (dose in mg/kg/day)			
	0	10	50	250	0	5	15	75
Body weight in grams ^{#1}	309 ±24 (19)	322 ±22 (20)	309 ±23 (20)	318 ±18 (20)	409 ±51 (20)	410 ±61 (20)	397 ±61 (20)	374 ±35 (19)
<u>Ovary</u> ^{#2}								
granulosa/thecal cell hyperplasia	0/20(0)	8/20(40)	19/20(95)	16/20(80)	0/20(0)	0/20(0)	0/20(0)	0/20(0)
atrophy	0/20(0)	0/20(0)	0/20(0)	0/20(0)	2/20(10) [1/12(8)]	4/20(20) —	5/20(25) —	6/20(30) 10/12(83)] ^{#3}
<u>Uterus</u> ^{#2}								
cystic glands	0/20(0)	0/20(0)	0/20(0)	0/20(0)	0/20(0) [0/12(0)]	3/20(15) —	3/20(15) —	3/20(15) 4/12(33)] ^{#3}
carcinoma	0/20(0)	0/20(0)	0/20(0)	0/20(0)	0/20(0) [0/12(0)]	0/20(0) —	0/20(0) —	0/20(0) 2/12(17)] ^{#3}

#1 : Mean ± SD, at week 26 and 52 in 6- and 12- month studies, respectively.

#2 : Number of animals with each finding / number of animals examined, (): %.

#3 : After the 24- week withdrawal period. —: not tested.

Table 4. Incidence of findings in the ovary and uterus of rats in the 2- year oncogenicity study.

Finding	Dose (mg/kg/day)			
	0	5	15	75
<u>Ovary</u>				
granulosa cell				
- adenoma	1/104(1.0)	1/52(1.9)	2/50(4.0)	2/52(3.8)
- carcinoma	1/104(1.0)	1/52(1.9)	0/50(0)	0/52(0)
<u>Uterus</u>				
polyp				
- stromal	18/104(17.3)	6/52(11.5)	7/52(13.5)	7/52(13.5)
- adenomatous	0/104(0)	1/52(1.9)	0/52(0)	1/52(1.9)
adenocarcinoma	0/104(0)	0/52(0)	1/52(1.9)	5/52(9.6)**
squamous carcinoma	0/104(0)	0/52(0)	0/52(0)	1/52(1.9)

Number of animals with each finding / number of animals examined, (): %.

** : P<0.01 (a trend test by the Peto method with multiplicity correction followed by pairwise comparison).

94.1%, respectively). No malignant Leydig cell neoplasms were observed. No significant tumor increase was observed in the mouse testis. Similar effects have been observed with other anti-androgens such as flutamide and cimetidine in rats (Winters *et al.*, 1979; FDA, US Summary Basis of Approval, Flutamide, 1980; Leslie *et al.*, 1981; Neumann, 1991). The 5α -reductase inhibitor, finasteride, has also been shown to cause Leydig cell tumors in rodents (Pralhada *et al.*, 1994). Studies using bicalutamide (50 mg/day) as monotherapy in metastatic prostate cancer patients showed no evidence of Leydig cell hyperplasia in clinical use (Jones *et al.*, 1994).

2. Female reproductive tract

There were changes recorded in the ovary and uterus of rats dosed with bicalutamide in the 6- and 12-month repeated dosing studies and the 2- year oncogenicity study. The findings and their incidences in these two organs are given in Tables 3 and 4.

The interactions of anti-androgens with ovarian and uterine androgen receptors are not

well understood. Androgens are synthesized by thecal cells of the ovary (Peluso, 1992). Granulosa/thecal cell hyperplasia observed in the six-month rat study may have resulted from intra-ovarian action at androgen receptors or indirectly via the pituitary-gonadal axis.

The rat uterus contains specific androgen receptors (Chang and Tindall, 1983) with many of the properties of the male androgen receptor. Activity at this receptor may account for the uterine pathology observed in rats in the 12-month study, in which cystic changes in endometrial glands were observed in all female dosed groups. This feature, together with associated ovarian granulosa/thecal cell hyperplasia, was indicative of pharmacological effects in females as well as males. Uterine changes in the 12-month and oncogenicity studies 75 mg/kg/day dose group showed progression to uterine adenocarcinoma at 18 months (after 24-week withdrawal period) and at 2 years, respectively, with only one case of adenocarcinoma in the 15 mg/kg/day dose group. These changes could be attributed directly to the pharmacological effects

Table 5. Adrenal gland weights and histological changes in 6- month study in rats.

Finding		Dose (mg/kg/day)			
		0	10	50	250
Body weight in grams at week 26 ^{#1}					
(Mean±SD)	Male	562±43 (19)	535±43 (19)	535±55 (18)	524±44* (19)
	Female	309±24 (19)	322±22 (20)	309±23 (20)	318±18 (20)
Adrenal gland weight in milligrams ^{#1}					
(Mean±SD)	Male	57±13 (19)	65±11 (19)	70±13** (18)	70±14*** (19)
	Female	84±13 (19)	91±13 (20)	101±38* (20)	94±9 (20)
Cortical hypertrophy ^{#2}					
	Male	0/20(0)	8/20(40)	11/20(55)	18/19(95)
	Female	1/20(5)	11/20(55)	16/20(80)	17/20(85)
Cortical vacuolation ^{#2}					
	Male	14/20(70)	11/20(55)	18/20(90)	17/19(89)
	Female	3/20(15)	3/20(15)	4/20(20)	5/20(25)

#1: The number in parenthesis shows the number of animals per group.

#2: Number of animals with each finding / number of animals examined, (): %.

* : P<0.05, **: P<0.01, ***: P<0.001 compared with the control group (ANOVA followed by Student's t-test).

of bicalutamide interfering with the pituitary-gonadal axis and an unopposed estrogen action on the uterus. No uterine tumors were observed in mice.

Development of uterine adenocarcinoma in neonatally androgen-sterilized or estradiol-treated rats (Morikawa *et al.*, 1982, Niwa *et al.*, 1991) was attributed to unopposed estrogen-dominant endocrine dysfunction of the endometrium. Similar endometrial changes in the bicalutamide oncogenicity study in the rat would point to endocrine disturbance as the basis for pharmacology-related uterine adenocarcinomas in the 75 mg/kg/day dose group.

Similar endocrine-related risk factors have been proposed in endometrial carcinoma in women (Lucas, 1974). Given the male-only indications for bicalutamide and the non-genotoxic nature of the compound, this finding in rats (but not seen in mice) is considered not to be of relevance to clinical usage in prostate cancer.

3. Adrenal effects

Table 5 shows the results of weight measurements and histological changes in the adrenal glands. In the multi-dose studies, increase in adrenal weight and the histological changes of increased female-pattern cortical hypertrophy/vacuolation are considered to be a pharmacological effect of treatment. The considerable sexual dimorphism of the adrenal cortex, especially in the rat is related to the hepatic production of corticosteroid binding globulin (CBG). This binding protein is increased in level by estrogens and suppressed by androgens; as a result, anti-androgen treatment will increase CBG levels, reduce free corticosteroid levels and, via stimulation of the pituitary-adrenal axis, result in the observed adrenal cortical enlargement. Although bicalutamide showed some binding to sex hormone binding globulin (SHBG), no binding to CBG occurred.

Enzyme-inducing effects and their consequences

1. Liver effects

Table 6 shows the survival rate and mean body weight at week 52, and Table 7 gives the incidence of hepatic tumors in oncogenicity studies. Bicalutamide, in common with many other drugs acting at steroid receptors, produces

hepatic enlargement in animals with associated hypertrophy as a consequence of induction of mixed function oxidase (MFO) enzymes of the cytochrome P450 family (Schulte-Hermann *et al.*, 1988; Mayol *et al.*, 1992). Biochemical and immunocytochemical studies in animals have provided evidence for MFO induction and increased cytochrome P450 isozymes CYP2B1 and CYP3A1 expression in the mouse and CYP3A1 expression in the rat. Enzyme induction was observed in the dog; this was phenobarbitone-like, as compared to phenobarbitone/dexamethasone-like in the mouse and dexamethasone-like in the rat. In contrast, studies of antipyrine clearance in human subjects have shown no effects of bicalutamide on human mixed function oxidase induction (Kaisary *et al.*, 1996). The steroidal anti-androgen cyproterone acetate (CPA) causes an increase in MFO activity in animal species (Brown *et al.*, 1976). The MFO-inducing potential of a third non-steroidal anti-androgen, nilutamide, in animals has not been reported. In the bicalutamide studies there were minor increases in cholesterol, alkaline phosphatase and alanineaminotransferase. These were considered to be consequences of the phenobarbitone-like enzyme-inducing effects in the liver after chronic administration of bicalutamide.

The small increase in incidence of hepatocellular carcinoma in male mice dosed at 75 mg/kg/day for 2 years can be attributed to a phenobarbitone-like non-genotoxic carcinogenic process. A comprehensive battery of *in vitro* and *in vivo* genotoxicity tests with bicalutamide was consistently negative. To investigate this further, a 3-month mouse study was conducted; it showed a classic phenobarbitone/dexamethasone-like induction of cytochrome P450 isozymes CYP2B1 and CYP3A1 (but not CYP1A2), centrilobular hypertrophy with increased smooth endoplasmic reticulum and increased cell proliferation. This process is identical to that described by McClain (1990) in several strains of mice. There was no evidence for peroxisome proliferation in the 3-month study, differentiating bicalutamide from the endogenous androgen dihydroepiandrosterone, which is also a rodent liver carcinogen (Rao *et al.*, 1992). Although the C57BL/6 mouse is refractory to

mouse liver tumor induction by phenobarbitone at 18 months (Becker, 1984), the C57BL/10J mouse used in this case is responsive to liver tumor induction by the non-genotoxic carcinogen and P450 enzyme inducer chlordane (Barrass *et al.*, 1993). CPA and pregnenolone have both been reported to increase ploidy in rat liver (Schulte-Hermann *et al.*, 1980) and both are MFO inducers active at steroid hormone

receptors. The restriction of tumor induction to male mice has been noted with a range of other non-genotoxic carcinogens (Ashby and Tennant, 1991; Grasso and Hinton 1991). There are no published oncogenicity results for the anti-androgen flutamide, but data have been published showing CPA to induce hepatocellular tumors in rats (Schuppler and Gunzel, 1976), unlike bicalutamide, and in mice (Foster *et al.*,

Table 6. Survival rate and mean body weight at week 104 in 2- year oncogenicity studies.

Species/Items		Dose (mg/kg/day)			
		0	5	15	75
<u>Rat</u>					
Survival rate (%)	Male	33	24	39	49
	Female	44	58	46	37
Mean body weight in grams at week 104	Male	551(37)	561(12)	507(21)*	501(25)**
	Female	396(49)	393(30)	367(24)*	358(21)**
<u>Mouse</u>					
Survival rate (%)	Male	47	40	42	42
	Female	50	50	48	44
Mean body weight in grams at week 104	Male	31.0(52)	29.8(22)*	30.2(21)	30.5(23)
	Female	25.7(51)	26.8(25)	26.5(23)	27.0(23)

*: P<0.05, **:P<0.01 compared with the control group (ANOVA followed by Student's *t*-test).

Table 7. Incidence of hepatic tumor in 2- year oncogenicity studies.

Lesion / dose (mg / kg / day)	Rats		Mice	
	male	female	male	female
Hepatocellular adenoma				
0	1/102(1.0)	1/104(1.0)	3/100(3.0)	0/100(0)
5	0/51(0)	1/52(1.9)	0/50(0)	0/50(0)
15	1/51(2.0)	1/51(2.0)	0/50(0)	0/50(0)
75	0/51(0)	1/52(1.9)	4/50(8.0)	0/50(0)
Hepatocellular carcinoma				
0	0/102(0)	1/104(1.0)	2/100(2.0)	0/100(0)
5	1/51(2.0)	0/52(0)	1/50(2.0)	1/50(2.0)
15	0/ 51(0)	0/51(0)	1/50(2.0)	0/50(0)
75	0/51(0)	0/52(0)	8/50(16.0)**	0/50(0)

Number of animals with hepatic tumors / number of animals examined, (): %.

** : P<0.01 (a trend test by the Peto method with multiplicity correction followed by pairwise comparison).

1993).

The maximum no-effect dose level for mouse liver neoplasia (15 mg/kg/day, blood level circa 13 $\mu\text{g/ml}$) was less than two fold above the human clinical exposure in Western patients receiving 50 mg/day and less than the exposure in Japanese subjects (18 $\mu\text{g/ml}$) receiving 80 mg/day, on a daily dose basis, but not a life-time cumulative dose basis. It should be remembered that survival duration for patients with advanced prostate cancer is limited even following established treatment. Mechanism and species difference factors rather than toxicokinetic parameters are also critical in extrapolating from mouse liver tumor evaluation to human risk.

2. Thyroid effects

The significant histopathological findings in thyroid glands are presented in Table 8. The increased thyroid follicular epithelial activity observed solely in rats can also be assigned as a secondary effect of enzyme induction in that species. Thyroid follicular stimulation in the rat typically results from either a direct goitrogenic action of a compound on the thyroid gland itself, e.g., by peroxidase inhibition, or an indirect effect due to increased clearance of thyroid hormone (Saito *et al.*, 1991; McClain, 1989). Increased ^{125}I -labelled thyroxine clearance was observed in rats given 75 mg bicalutamide/kg/day at 28 days, confirming an indirect mode of action of this compound, analogous to that seen with SKF93479 (Brown *et al.*, 1987) and phenobarbital (Oppenheimer *et al.*, 1968). Thyroid stimulating hormone (TSH) was only slightly increased ($P > 0.05$) but this was probably due to the experimental design, because the

histology was typical of raised TSH. Increased thyroid hormone clearance with associated liver enlargement therefore provides an explanation of the thyroid hypertrophy, although the precise biochemical pathway was not elucidated.

The 2-year rat oncogenicity study showed a dose-related increase of thyroid follicular adenoma (benign), with an increased frequency in all dose groups. This tumor is considered to be a secondary effect of MFO induction and associated hepatic liver enlargement leading to increased thyroid hormone clearance and consequent TSH stimulation of the thyroid gland. The induction of thyroid follicular adenoma (also seen in the 12-month rat study) is, therefore, correlated with liver hypertrophy and MFO induction in the rat; hence, the presence of adenoma at the lower dose of 5 mg/kg in the 2-year oncogenicity study is an expected effect.

As with other liver-enlarging MFO inducers, the formation of thyroid tumors by the mechanism described in the rat does not constitute a risk for man (Hill *et al.*, 1989; McClain, 1989). The absence of thyroid tumors at the end of the withdrawal period in the 12-month toxicity study, would also support a hormonal basis for tumor development, as does the lack of similar effects in the mouse and the dog.

Other toxicities

1. Hematological changes

Minor changes to erythrocyte parameters (e.g., decreases in hemoglobin, hematocrit and calculated red cell indices) seen in rats were attributable to pharmacological actions opposing the stimulatory action of androgen on

Table 8. Thyroid follicular cell adenoma in 2- year oncogenicity studies.

Dose (mg/kg/day)	Rats		Mice	
	male	female	male	female
0	4/101(4.0)	0/103(0)	8/99(8.1)	0/98(0)
5	9/50(18.0)**	2/49(4.1)	0/48(0)	1/49(2.0)
15	16/51(31.4)***	8/49(16.3)***	0/50(0)	1/48(2.1)
75	29/51(56.9)***	22/52(42.3)***	7/50(14.0)	2/50(4.0)

Number of animals with thyroid follicular cell adenoma / number No. of animals examined, (): %.

** : $P < 0.01$, *** : $P < 0.001$ (a trend test by the Peto method with multiplicity correction followed by pairwise comparison).

hematopoiesis.

A feature of the 6-month dog study was an outbreak of clinical disease, with one mid-dose and one high-dose male dog being killed prematurely because of their poor clinical condition, and one high-dose female showing significant clinical signs before recovering spontaneously. No specific pathogen was identified; however, the associated neutropenia, followed by a regenerative bone marrow response (which recovered during further dosing), and the failure to reproduce the finding in the subsequent 12-month study, point to a background disease as the cause, rather than a toxic effect of the compound. No treatment-related changes in neutrophil or total leukocyte numbers were observed in clinical trials (Kotake *et al.*, 1996a; Kotake *et al.*, 1996b).

2. Cardiac changes

Only the electrocardiographic abnormalities (reduced P-R interval) in bicalutamide-treated dogs are of unexplained etiology. This change was observed down to a dose of 2.5 mg/kg in the 6-month dog study. In this dose group, steady-state plasma concentrations for total bicalutamide of 25 µg/ml were achieved. However, the extent and severity of the changes were small and lay within the normal range for the laboratory. There were no histopathological changes in the heart even at the highest dose (100 mg/kg/day). Similar changes were recorded with higher doses in short-term general pharmacology studies. A maximum no-effect dose for the electrocardiographic finding of reduced P-R interval was established at 1 mg/kg in the 12-month study. Western subjects treated with up

Table 9. Effects on fertility in dosed male rats (F0).

Items	Dose (mg/kg/day)			
	0	1	10	250
Body weight in grams ^{#1}	541±40(25)	540±41(25)	547±32(25)	528±37(25)
Pre-coital interval (days) ^{#1}				
First pairing	2.2±1.6(25)	1.9±1.6(25)	2.6±3.0(25)	6.1±5.0(25)***
Second pairing	2.1±1.0(25)	2.0±1.1(25)	2.4±1.3(25)	2.4±2.2(25)
Third pairing	1.9±1.1(25)	2.5±2.3(24)	2.2±1.4(24)	1.8±0.8(24)
Time to successful mating (days) ^{#1}				
First pairing	2.7±2.3(25)	2.8±2.9(24)	7.7±10.1(25)	14.4±8.4(25)***
Second pairing	2.6±2.8(25)	2.1±1.0(25)	2.4±1.3(25)	2.4±2.2(25)
Third pairing	3.0±5.3(25)	2.5±2.3(24)	2.0±1.5(22)	2.8±4.8(24)
Copulation index ^{#2}				
First pairing	25/25(100)	25/25(100)	25/25(100)	25/25(100)
Second pairing	25/25(100)	25/25(100)	25/25(100)	25/25(100)
Third pairing	25/25(100)	24/24(100)	25/25(100)	24/24(100)
Fertility index ^{#3}				
First pairing	25/25(100)	24/25(96)	25/25(100)	25/25(100)
Second pairing	25/25(100)	25/25(100)	25/25(100)	25/25(100)
Third pairing	25/25(100)	24/24(100)	23/25(92)	24/24(100)

Times: first pairing, the last day of dosing ; second pairing, 7 weeks after the last day of dosing; third pairing, 16 weeks after the last day of dosing.

#1: Mean ± SD. The number in parenthesis shows the effective number of animals.

#2: Number of animals mated / number of animals paired, (): %.

#3: Number of fertile animals / number of animals mated, (): %.

*** : P<0.001 compared with the control group (ANOVA followed by Student's t-test)

to 200 mg/day were subjected to detailed ECG monitoring with no evidence for treatment-related ECG abnormalities being detected (Kolvenbag and Blackledge, 1996).

3. Genetic toxicology studies

A comprehensive battery of *in vitro* and *in vivo* tests has been employed.

Bacterial mutagenicity studies conducted with 5 strains of *Salmonella typhimurium* (TA100, TA1535, TA1537, TA1538 and TA98) and 2 strains of *Escherichia coli* (WP2 and WP2 *uvra* pK101), in the presence and absence of S9 mix, were all negative up to the maximum (limit) dose of 2000 μ g/plate. Bicalutamide also gave negative results in a mutagenicity study using the yeast *Saccharomyces cerevisiae* strain D7. The maximum dose level tested was 500 μ g/ml, which was the maximum solubility of the compound in the test system. A mammalian cell mutagenicity assay with Chinese hamster ovary cells was also negative up to dose levels causing cytotoxicity, as determined by cell survival rate (29% in the absence of S9 and 14% in the presence of S9).

Both *in vitro* and *in vivo* chromosome aberration assays were conducted. The *in vitro* study used cultured human lymphocytes from two donors and gave negative results up to dose levels that caused either a decrease in mitotic index or poor chromosome morphology. The *in vivo* study examined cells from the bone marrow of male and female rats at two sampling times after a single oral dose of 2000 mg/kg (limit dose). There was no effect on the incidence of chromosome damage either 12 or 24 hours after dosing.

The micronucleus test was conducted in the mouse at the limit dose of 2000 mg/kg. The bone marrow was sampled 24, 48 and 72 hours after dosing in both male and female animals. Bicalutamide did not affect the incidence of micronucleated polychromatic erythrocytes, and was therefore negative in this assay.

4. Reproduction toxicology studies

The potential to cause reproductive toxicity has been assessed following oral bicalutamide administration in the rat and rabbit. Given the anti-androgen activity of bicalutamide in all species tested, significant interference with the male reproductive behavior, reproductive tract function and fertility, and androgen-dependent

differentiation of the fetus and neonate were to be expected. The male reproductive study was, therefore, substantially modified to allow for a recovery period. As shown in Table 9, normal mating behavior, or at least vaginal plug formation, returned 7 weeks after the end of an 11-week dosing period, which was sufficient to ensure exposure at all stages of spermatogenesis and sperm maturation. Following restoration of mating in the male rat, reduced fertility was observed at 5 and 250 mg/kg/day on first mating, but this returned to normal on subsequent matings. Implantation and fetal development were unaffected.

There was no evidence for any developmental or teratogenic effect in the rabbit following treatment of dams. In the rat no abortifacient or teratogenic (except pharmacological) effects were seen except for a predictable reduction of ano-genital distance in male fetuses which was seen at 10 mg/kg and above when assessed at day 20 of pregnancy (Table 10). No effects were seen on this parameter in rats dosed at 1 mg/kg/day. Comparable changes were observed in studies with other anti-androgens e.g., flutamide (FDA, US Summary Basis of Approval. Flutamide, 1980).

Male offspring in the Segment III reproductive study showed hypospadias (feminization of male fetus with failure of urethral closure) with a subsequent predisposition for ascending urinary tract infections (Table 10). Male fertility and reproductive performance was severely impaired in the 10 and 250 mg/kg/day dose groups with no female being successfully impregnated. Subsequent mating of females from these two groups with proven control males revealed a reduction in fertility (Table 11). Although not assessed, hypothalamic imprinting by testosterone would also be expected to be blocked if bicalutamide crossed into the hypothalamus at this stage of development. Prominent nipple development in male offspring was also observed following bicalutamide treatment. Similar changes have been reported following treatment with flutamide (Tate-Ostroff and Bridges, 1988). Transplacental feminization of the male fetus was not observed in rabbits; the basis of this species difference is unknown.

Table 10. Effects on male fetuses/offspring rats (F₁).

Items	Dose (mg/kg/day)				
	0	0.1	1	10	250
Body weight					
Seg II (offspring)	—	—	—	—	—
Seg III (offspring)	—	—	—	—	↓
Ano-genital distance (mm)					
Seg II (fetuses) ^{#1}	4.40(22)	NT	4.33(22)	4.04(20)***	3.90(21)***
Seg II (offspring) ^{#2}	4.19(8)	4.07(12)	4.17(10)	3.90(10)***	3.76(11)***
Seg III (offspring) ^{#2}	4.40(19)	4.37(19)	4.29(18)	3.44(18)***	3.20(23)***
Hypospadia (%) ^{#3}					
Seg III (offspring)	0/22(0)	0/22(0)	0/22(0)	17/22(72)	22/22(100)
Copulation index ^{#4}					
Seg II (offspring)	12/13(92)	12/12(100)	13/13(100)	13/13(100)	12/13(92)
Seg III (offspring)	21/22(95)	22/22(100)	21/22(95)	6/21(29)	2/22(9)
Fertility index ^{#5}					
Seg II (offspring)	12/12(100)	11/12(92)	13/13(100)	13/13(100)	11/12(92)
Seg III (offspring)	18/21(86)	20/22(91)	15/21(71)	0/6(0)	0/2(0)

NT: not tested, —: no significant change, ↓: significant reduction.

#1: Day 20 of pregnancy. The data for the 50 mg/kg group (n=21) are omitted because parameters except ano-genital distance (4.03***) were not tested.

#2: Day 1 of lactation.

#3: 36 days after birth. Number of animals with hypospadia / number of animals examined, (): %.

#4: Number of animals mated / number of animals paired, (): %.

#5: Number of fertile animals / number of animals mated, (): %.

***: P<0.001 compared with the control group (ANOVA followed by Student's t-test).

Table 11. Effects on female offspring rats (F₁).

Items	Dose (mg/kg/day)				
	0	0.1	1	10	250
Body weight					
Seg II	—	—	—	—	—
Seg III	—	—	—	—	—
Copulation index ^{#1}					
Seg II	13/13(100)	12/12(100)	13/13(100)	13/13(100)	13/13(100)
Seg III	22/22(100)	22/22(100)	21/22(95)	20/22(91) ^{#3}	21/22(95) ^{#3}
Fertility index ^{#2}					
Seg II	13/13(100)	11/12(92)	13/13(100)	13/13(100)	13/13(100)
Seg III	21/22(95)	22/22(100)	20/21(95)	16/20(80) ^{#3}	15/21(71) ^{#3}

—: no significant change.

#1: Number of animals mated / number of animals paired, (): %.

#2: Number of fertile animals / number of animals mated, (): %.

#3: Results of the mating with fertile males in the control group.

The data for the 50 mg/kg0mg/kg/day group are omitted because not all parameters were tested.

CONCLUSION

Bicalutamide is essentially non-toxic at all dose levels studied in animals, with only pharmacological and adaptive enzyme induction effects being observed. A reversible sub-clinical change in the electrocardiogram (reduction in P-R interval) was also seen. The maximum no-effect dose for this latter change was 1 mg/kg/day in the 12-month dog study. No treatment-related ECG changes have been observed in bicalutamide-treated patients.

As an anti-androgen, bicalutamide produced pharmacological changes in the male fetus/offspring of the rat, including feminization of the male fetus/offspring, such as decreased ano-genital distance and hypospadias. No effects were seen in the female rat fetus or in the rabbit fetus. Effects on male and female fertility and reproductive tract histology were related to anti-androgen activity.

There was no evidence of mutagenicity or clastogenicity in a comprehensive battery of *in vitro* tests in bacterial, yeast and mammalian cell systems and *in vivo* tests in rats and mice.

A non-genotoxic mechanism (associated with LH feedback and enzyme induction) for the formation of benign Leydig cell and thyroid follicular cell adenomas in the rat and hepatocellular carcinoma in male mouse is supported by mechanistic studies.

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