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Effects of Cimetidine, Progesterone, Cannitracin and Tolazoline on the Weight and DNA Content of the Testosterone-Induced Hyperplastic Prostate of the Rat

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Summary. The antiandrogenic potency of cimetidine, progesterone, cannitracin and tolazoline was studied in intact immature rats by determination of the weight and DNA content of the ventral prostate. Cimetidine is a H-2-recepter histamine antagonist, cannitracin is an antifungal antibiotic derived from Strp.griseus and tolazoline is an α-adrenergic blocker similar to phentolamine. All rats (except the control group) received testosterone propionate (TP) 0.3 mg s.c. with the drugs above every day for one week. Cimetidine, progesterone and cannitracin, but not tolazoline, significantly decreased the weight and DNA content of the hyperplastic prostate induced by TP. The results indicate that cimetidine, progesterone and cannitracin have an antiandrogenic effect. This study provides the basis for investigating the effects of antiandrogen in men with symptomatic benign prostatic hyperplasia.

Key words: Cimetidine – Progesterone – Cannitracin – Tolazoline – Benign prostatic hyperplasia

Introduction

Despite the excellent results obtained by operative measures in most patients with benign prostatic hyperplasia, a satisfactory method of medical treatment has occupied the attention of many research workers [1]. Although the exact physiopathological factors that produce prostatic hyperplasia (BPH) are not fully understood it has been hypothesized that at least one important factor may be the androgenic effect of testosterone derivatives, especially 5α-dihydrotestosterone, on the prostatic tissue [2, 3].

Consequently one logical way to attempt to influence this would be by use of antiandrogenic compounds. A number of these compounds have been reported for curing BPH, such as H-2-receptor histamine antagonist:cimetidine [4], progestational agents:hydroxyprogesterone [5, 6] and ployene macrolis, which includes amphotericin B and candicidin [7, 8, 9, 10]. Our study has been designed to evaluate antiandrogenic potency of cimetidine, progesterone, cannitracin and tolazoline by determination of the weight and DNA content of rat ventral prostate, by precise and reproduciable means [11, 12].

Materials and Methods

Animals

Immature male albine rats of the Spragure-Dawley strain (66–120 g) were weighed and randomly distributed into treatment groups consisting of six to twenty animals. Twenty-four hours after the final injection, the rats were killed and the sex organs were removed and chilled. The ventral prostate was weighed, some of the prostate was removed and immediately immersed in 4% neutral buffered formalin for histologic studies, and the remainder was stored for biochemical analysis.

Drugs injection

Testosterone propionate (TP) was dissolved in sesame oil at a concentration of 0.3 mg per 0.2 ml. Cannitracin was given by gavage and the rest of drugs were injected subcutaneously at independent sites. Nomenclature of the compounds referred to in this study are recorded in Tables 1 and 2.

Determination of DNA content

According to the Deklerk's method [13], after the removal of tissue for histologic processing, the tissue was reweighed and chilled. All further steps were carried out at 0 to 2 °C. The tissue was minced to a fine pulp with opthalmologic sissors and suspended in 5 ml of 0.4 M perchloric acid (PCA). The tissue suspension was centrifuged at 1,000 × g for 10 min at 4 °C. The pellet was washed twice with 5 ml of 0.2 M PCA. The RNA was then removed from the washed pellet by alkaline hydrolysis with 4 ml of 0.3 M KOH at 37 °C for 2 h. After reprecipation with 6 ml of 1.6 M PCA, the DNA in the pellet was acid hydrolyzed by adding 2 ml of 1.6 M PCA and heat-

Table 1. Nomenclature of the compounds referred to in this study

Code letter	Common name	Chemical name	Molecular wt	Manufacturer
A	Testosterone Propionate	Δ^4-Androsten-17β-propionate-3-one	344	Shanghai No. 9 Pharmaceutical Factory
B	Cimetidine	1-Methyl-3-[2-(5-methylimidazol-4-ylmethyl thio)ethyl]guanidine-2-carbonitrile	240	Shanghai No. 1 Pharmaceutical Factory
C	Progesterone	4-pregnene-3,20-dione	314	the same as A
D	Cannitracin			Zhejiang Haimen Pharmaceutical Factory
E	Tolazoline	2-Benzyl-2-imdazoline	160	Shanghai No. 13 Pharmaceutical Factory

Table 2. 109 rats were distributed into seven groups

Code letter	Groups	Dose (mg/day/rat)	Number of rats	Days
1	Control	0.2 ml sesame oil	17	7
2	TP	0.3	16	7
3	TP + Cimetidine	0.3 + 20	20	7
4	TP + Progesterone	0.3 + 10	18	7
5	TP + Cannitracin	0.3 + 10	16	7
6	TP + Tolazolline	0.3 + 7.5	16	7
7	TP + Cimetidine + Cannitracin	0.3 + 20 + 10	6	7

Table 3. Effect of the drugs on the weight of the ventral prostate (VP) and seminal vesicle (SV) of the rat induced by TP

Groups	Number of rats	Wet weigt (mg/100 g · BW) ± SD	
		VP	SV
1	17	73.1 ± 16.9	38.5 ± 11.9
2	16	139.7 ± 15.2	131.3 ± 17.7
3	20	105.8 ± 18.2**	108.4 ± 20.5**
4	18	128.1 ± 15.8*	127.6 ± 16.5
5	16	128.6 ± 14.7*	112.7 ± 12.5**
6	16	138.2 ± 16.9	135.4 ± 23.1
7	6	107.3 ± 15.9**	115.4 ± 13.7*

* $P < 0.05$; ** $P < 0.01$ vs. Group 2

Table 4. Comparison of the effect of antiandrogens on the rat ventral prostate DNA polymerase activity (X ± SD)

Groups	Number of rats	Total DNA (μg/100 g · BW)	Total RNA (μg/100 g · BW)	RNA/ DNA
1	17	162.9 ± 15.5	375.8 ± 27.9	2.3
2	16	272.4 ± 29.3	535.9 ± 11.7	2.0
3	20	206.5 ± 30.7**	475.6 ± 25.5**	2.2
4	18	251.6 ± 25.6	526.9 ± 19.6*	2.1
5	16	247.3 ± 26.7*	523.5 ± 19.2*	2.1
6	16	283.5 ± 28.0	535.3 ± 24.0	1.9
7	6	187.2 ± 22.0**	443.4 ± 17.4**	2.4

* $P < 0.05$; ** $P < 0.01$ vs. Groups 2

ing for 20 min at 70 °C. The hydrolyzed DNA suspension was cooled and centrifuged at 1,000 x g for 10 min at 0 °C. The supernatant was removed for DNA analysis by reformed-diphenlamine method with nucleoprotamine DNA as the reference material. RNA content was assayed by orcinol reaction with yeast RNA as standard. Du-7uv-visible spectrophotometer (Beckman, USA) was used for this assay.

Results

Effects of cimetidine, progesterone, cannitracin and tolazoline on the weight of the hyperplastic prostate and seminal vesicle induced by TP are shown in Table 3. The weight of the ventral prostate and seminal vesicle of the rat receiving cimetidine were markedly decreased compared with those of rat given TP alone ($P < 0.01$). The weight of the prostate of the rat receiving progesterone and cannitracin was decreased ($P < 0.05$). The weight of prostate treated with cimetidine in combination with cannitracin was decreased significantly ($P < 0.01$). Effect of cimetidine, progesterone, cannitracin and tolazoline on the nucleic acid is shown in Table 4. RNA and DNA content was significantly decreased in the prostate of rats receiving cimetidine compared with those of rats given TP alone ($P < 0.01$). RNA and DNA were also decreased in the prostate of the rat treated with cannitracin ($P < 0.05$). RNA content was decreased in the prostate of the rat given progesterone ($P < 0.05$).

Discussion

Antiandrogens have been proposed as a new series of compounds which might prove useful in the control of abnormal growth of the human prostate [14, 15]. Generally, antiandogens produce their effect by direct competition with androgens at the target orgen site and can directly inhibit the effect of exogenous testosterone in inducing growth of accessory sex tissue in male rats [12].

Present bioassay models for evaluating the potency of antiandrogen involve the effect of these drugs on: (i) the androgen-induced growth in chick comb; (ii) the androgen-induced increase in the wet weight of the accessory sex tissue in male rodents; (iii) changes in the volume of canine prostatic secretion collected through prostatic fistula preparations [16, 17]. Changes in DNA are directly related to increase in cell number while other methods utilize changes in tissue wet weight, which might be affected by glandular secretion and water retention. We used the content of RNA and DNA as an index of prostatic growth which had the advantage of being precise and reproducible [12].

Cimetidine is an H-2-receptor blocker and some reports of cimetidine-associated gynecomastia have appeared in the literature [18, 19]. In 1977, Leslie et al. [20] stated the weight of prostate and seminal vesicle could be decreased in both the rat and in the dog by the administration of cimetidine. Winters et al. confirmed that this drug was a nonsteroidal antiandrogen. More recently, Tang [4] reported improvement in patients with BPH treated with cimetidine. Our experiment showed that cimetidine could decrease the weight and DNA content of the prostate of the rat induced by TP, compatible with previous observation.

The antiandrogenic effect of progesterone is well known. Progesteronal agents have been used in the treatment of BPH [5, 6]. The action of progesterone probably could be reducing pituitary gonadotropin secretion [21]. In animals it is possible to reduce growth of the prostate gland by modifying the hormonal state of these animals [22]. In our experiment, progesterone could decrease the weight of the prostate and seminal vesicle and RNA content. These results demonstrated that progesterone has antiandrogenic effect.

Candicidan was isolated from a strain of streptomyces griseus. In common with amphotericin B and nystatin it is an effective agent against pathogenic yeast and fungal organisms. In 1968, Gordon and Schaffner [23] found that oral administration of these compounds resulted in a reduction in size and a softening of the prostate gland of male dogs. Histological studies suggested that these effects were due to a reduction in the glandular component of the hypertrophied prostate. The efficacy of candicidin to treat BPH is controversial. Some authors reported a significant improvement following candicidin treatment [7], others found no effect [8]. Cannitracin is equivalent to candicidin in structure. Some reports the effects of cannitracin were conflicting [9, 10], but from our results this drug has an antiandrogenic potency which decreased the weight and DNA content of the accessory sex organs of the rat. Certain studies showed that the drug reduced the plasma cholesterol level of chicks and chickens by interference with exogenous cholesterol absorption, thereby altering cholesterol transport kinetics [23, 24].

More recently, many reports about the role of alpha-adrenergic blockers in the treatment of BPH appeared. After administration of this kind of the drug, the obstruction signs and symptoms were reduced. Our experiment failed to prove that tolazoline had any antiandrogenic effect. Many studies have shown that in BPH, the smooth muscle in the adenoma and prostatic capsule were rich in alpha-adrenergic receptor [25, 26]. The response of the smooth muscle in the adenoma and capsule of the prostate to stimulation of its alpha-adrenergic receptors consists of a contraction or an increase in tone. Therefore variation in the vivo owing to either intrinsic or extrinsic causes will result in corresponding variation in the tone of the prostate and capsular muscle. It was logical to suggest that by blocking the alpha-adrenergic receptors with an appropriate pharmacological agent, one could hope to abolish the superadded dynamic component and leave the patient with the basic degree of obstruction produced by the mechanical component alone [27].

Conclusion

Cimetidine, progesterone and cannitracin decreased the weight and DNA content of hyperplastic prostate induced by TP. The results indicate that these drugs have an antiandrogenic effect and could be used to improve some cases of symptomatic BPH. The therapeutic effect of alpha-adrenergic blockers on BPH is not because of any antiandrogenic effect.

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