J. steroid Biochem. Vol. 36, No. 3, pp. 221–223, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

EFFECT OF TOREMIFENE ON ESTROGEN PRIMED VAGINAL MUCOSA IN POSTMENOPAUSAL WOMEN

J. MÄENPÄÄ,[1,3]* K.-O. SÖDERSTRÖM,[2,3] M. GRÖNROOS,[1,3] E. TAINA,[1,3] A. HAJBA[3] and L. KANGAS[3]

[1]Departments of Obstetrics and Gynecology and [2]Pathology, University of Turku and [3]Farmos Group Ltd, Research Center, SF-20101 Turku, Finland

Summary—The antiestrogenic effect of 20 mg toremifene daily for 7 days and 68 mg for 5 days was studied in postmenopausal women volunteers primed for 7 days with estradiol valerate (2 mg daily orally) which was continued throughout the study. A control group received estrogen only and a reference group estrogen with 60 mg tamoxifen for 5 days.

No treatment opposed the action of the estrogen on the endometrium but both 68 mg toremifene and 60 mg tamoxifen statistically significantly decreased the maturity index of vaginal cells on day 13. A decrease was also evident on day 18 with 20 mg toremifene.

INTRODUCTION

Tamoxifen has for years been the only widely-used antiestrogen for hormonal control of endocrine-related tumors [1]. Although safe and selective in effect when compared with classical cytotoxic drugs, tamoxifen has been reported to induce hyperplastic nodules in the rat liver at high doses [2]. Similarly liver damage in human patients [3] and exacerbation of several side effects have been described with doses higher than 40 mg daily, without any increase in the antitumor effect [4].

The search for more effective and even more safe antiestrogens than tamoxifen has recently yielded a new compound, toremifene, chemically 4-chloro-1,2-diphenyl-1-(4(2-(*N*,*N*-dimethyl-amino)ethoxy)-phenyl)-1-butene [5, 6]. Although quite similar in effect to tamoxifen, toremifene has been found to have certain unique properties: (1) there are differences in the turnover rate of the unclear estrogen-receptor complex [5], (2) toremifene is less toxic in rats than tamoxifen, the safety ratio of lethal dose in subacute toxicity being 2–4, and consequently toremifene can be given in higher doses [6], (3) estrogen-receptor negative murine uterine sarcoma responds to toremifene but not to tamoxifen [6]. Based on these findings clinical studies with toremifene were started. Toremifene was found to be a very well tolerated compound up to the dose of 680 mg daily in the first dose tolerance studies. Toremifene had no effects on hematology or for example on liver function [7]. The aims of the present study were to investigate the ability of toremifene to oppose the effect of estradiol on vaginal epithelium as compared to tamoxifen and to find the minimal antiestrogenic dose of toremifene in humans.

PATIENTS AND METHODS

23 voluntary, postmenopausal, healthy (except for mild or moderate uterine prolapse) women participated in the study. Their mean (±SD) age was 65.4 ± 6.6 yr and the mean interval after the menopause was 15.3 ± 7.7 yr.

The design of the study is illustrated in Fig. 1. The hormonal parameters studied included the serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone-binding globulin (SHBG, excluding 3 persons) and estradiol. The samples for vaginal cytology and endometrial histology were interpreted by one of the authors (K.-O.S.). The cytological samples were processed according to the original method of Papanicolau and

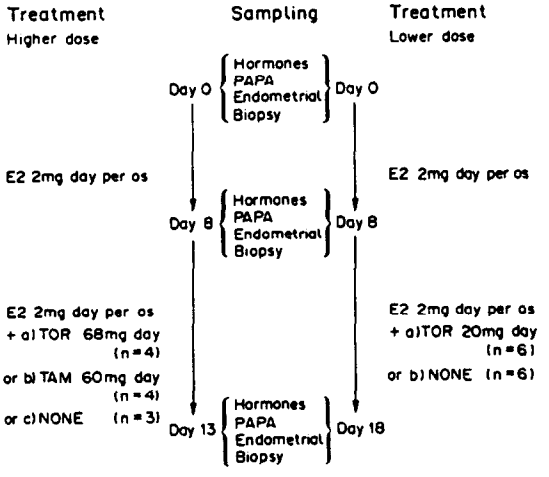

Fig. 1. The design of the study. E2 = estradiol, TAM = tamoxifen, TOR = toremifene.

Proceedings of the Toremifene Satellite Symposium held at the *UICC World Cancer Congress*, Budapest, Hungary, 1986.

*To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Turku University Central Hospital, Kiinamyllynkatu 4-8, SF-20520 Turku, Finland.

Table 1. The maturity indices and the observed changes in the numbers of superficial cells in human vaginal epithelium

Maturity index								
Toremifene (68 mg daily)			Tamoxifen (60 mg daily)			No antagonist		
Subject	Day	Index	Subject	Day	Index	Subject	Day	Index
LS	0	0/98/2	LK	0	0/90/10	LL	0	0/80/20
	8	0/55/45		8	0/52/48		8	0/55/45
	13	0/75/25		13	0/70/30		13	0/30/70
AS	0	40/55/5	MP	0	0/95/5	TT	0	100/0/0
	8	0/90/10		8	0/68/32		8	0/80/20
	13	0/67/33		13	0/72/28		13	0/40/60
EM	0	15/80/5	SV	0	100/0/0	KH	0	0/100/0
	8	0/70/30		8	0/58/42		8	0/65/35
	13	0/60/40		13	0/75/25		13	0/60/40
TK	0	2/98/0	TH	0	0/90/10			
	8	0/60/40		8	0/70/30			
	13	0/90/10		13	0/75/25			

Percentage change of the superficial cells										
Toremifene				Tamoxifen				No antagonist			
	Days				Days				Days	
Subject	0–8	8–12	0–12	Subject	0–8	8–12	0–12	Subject	0–8	8–12	0–12
LS	+43	−20	+23	LK	+38	−18	+20	LL	+25	+25	+50
AS	+5	+23	+28	MP	+27	−4	+23	TT	+20	+40	+60
EM	+25	+10	+35	SV	+24	−17	+25	KH	+35	+5	+40
TK	+40	−30	+10	TH	+20	−5	+15				
Mean	+28	−4	+24*		+32	−10*	+21*		+27	+23	+50
SD	17	25	9		10	8	4		8	18	10

Estradiol, 2 mg daily was given to all subjects on days 1–12. Toremifene 68 mg, tamoxifen 60 mg or no estrogen antagonist were given daily on days 8–12.
*$P < 0.05$ when compared to no antagonist treatment.

the histological samples according to standard light microscopical methods. The endometrial biopsies were taken only in the higher dose level (68 mg toremifene, 60 mg tamoxifen), study.

The statistical analyses were performed with Student's t-test.

RESULTS AND DISCUSSION

During estradiol priming the values of serum LH and FSH decreased and those of SHBG and estradiol increased. The addition of toremifene or tamoxifen had no effect on these levels.

Endometrial biopsies showed that neither 68 mg toremifene nor 60 mg tamoxifen could stop the proliferation of the endometrial cells induced by 2 mg estradiol within the 5-day treatment period. This period may not be sufficiently long to observe any antiestrogenic effect on the endometrium.

The maturity indices of the vaginal epithelium in the groups given 68 mg toremifene or 60 mg tamoxifen and estradiol and the mean changes in the proportion of superficial cells are presented in Table 1. Both toremifene and tamoxifen could significantly oppose the effect of estradiol on the vaginal epithelium. The drugs did not differ statistically significantly from each other. At 20 mg toremifene daily for 10 days (Table 2), there was still a reduction, which however was not statistically significant, in the number of superficial cells compared to the stimulation observed with estradiol alone. Since the half-life of toremifene is about 5–6 days and roughly half the

Table 2. The maturity indices and the observed changes in the numbers of superficial cells in human vaginal epithelium

Maturity index					
Toremifene (20 mg daily)			No antagonist		
Subject	Days	Index	Subject	Days	Index
RN	0	100/0/0	AA	0	0/100/0
	8	0/90/10		8	0/60/40
	18	0/68/32		18	0/60/40
EL	0	0/80/20	VJ	0	100/0/0
	8	0/35/65		8	0/70/30
	18	0/70/30		18	0/70/30
JN	0	0/98/2	JA	0	0/90/10
	8	0/85/15		8	0/80/20
	18	0/75/25		18	0/70/30
DB	0	100/0/0	AP	0	100/0/0
	8	0/70/30		8	40/50/10
	18	0/80/20		18	0/52/48
KR	0	100/0/0	LN	0	100/0/0
	8	0/80/20		8	5/80/15
	18	0/80/20		18	0/85/15
EJ	0	90/10/0	RH	0	0/55/45
	8	0/85/15		8	0/90/10
	18	0/80/20		18	0/45/55

Percentage change of the superficial cells							
Toremifene (20 mg)				No antagonist			
	days				days		
Subject	0–8	8–18	0–18	Subject	0–8	8–18	0–18
RN	+10	+22	+32	AA	+40	0	+40
EL	+45	−35	+10	VJ	+30	0	+30
JN	+13	+10	+23	JA	+10	+10	+20
DB	+30	−10	+20	AP	+10	+38	+48
KR	+20	0	+20	LN	+15	0	+15
EJ	+15	+5	+20	RH	−35	+45	+10
Mean	+22	−1	+21		+12	+16	+27
SD	13	20	7		29	21	15

Estradiol, 2 mg daily was given to all subjects on days 1–18. Toremifene 20 mg or no estrogen antagonist were given daily on days 8–17.

steady-state serum concentration is reached after 10 days, it may be possible to decrease the toremifene dose even further on long-term treatment. Studies in this direction are ongoing.

REFERENCES

1. Baum M. and Berstock D.: Tamoxifen as an adjuvant agent. *Clinics Oncol.* **1** (1982) 908–911.
2. Watanabe M., Tanaka H., Koizumi H., Tanimoto Y., Torii R. and Yanagita T.: General toxicity of studies of tamoxifen in mice and rats. *Jitchuken Zenrinsko Kenkyuko* **61** (1980) 1–36.
3. Blackburn A. M., Amiel S. A., Mills R. R. and Rubens R. D.: Tamoxifen and liver damage. *Br. Med. J.* **289** (1984) 288.
4. Tormey D. C., Lippman M. E., Edwards B. K. and Cassidy J. G.: Evaluation of tamoxifen doses with and without fluoxymestrone in advanced breast cancer. *Ann. Intern. Med.* **98** (1983) 139–144.
5. Kallio S., Kangas L., Blanco G., Johansson R., Karjalainen A., Perilä M., Piippo I., Sundquist H., Södervall M. and Toivola R.: A new triphenylethylene compound, Fc-1157a. I. Hormonal effects. *Cancer Chemother. Pharmac.* **17** (1986) 103–108.
6. Kangas L., Nieminen A-L., Blanco G., Grönroos M., Kallio S., Karjalainen A., Perilä M., Södervall M. and Toivola R.: A new triphenylethylene compound, Fc-1157a. II. Antitumor effects. *Cancer Chemother. Pharmac.* **17** (1986) 109–113.
7. Kivinen S. and Mäenpää J.: Effects of toremifene on clinical chemistry, hematology and hormone levels at different doses in healthy postmenopausal volunteers: phase I study. *14th UICC Int. Cancer Congr.*, Budapest, August 21–27, 1986. Karger, Budapest (1986) p. 778.