INHIBITION OF ESTROGEN SYNTHESIS IN HUMAN BREAST TUMORS BY TESTOLOLACTONE AND BROMOANDROSTENEDIONE

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ABSTRACT

The inhibition of aromatase enzyme in human breast tumors by Δ^1-testololactone, testololactone, 6α-bromoandrostenedione, and 6β-bromoandrostenedione was investigated. Estrone and estradiol synthesis from androstenedione was reduced in 3 tumor incubations by the presence of 0.13 mM Δ^1-testololactone and testololactone. 6α- and 6β-bromoandrostenedione (2.0 μM) were also shown to block estrogen synthesis in 2 tumors. Furthermore, Lineweaver-Burk plots revealed that all 4 compounds are competitive inhibitors of androstenedione aromatization. An apparent K_m of the aromatase enzyme for androstenedione of 0.08 μM and a V_{max} of 23 pmol of estrone synthesized/g tumor/hr were determined for one human breast tumor specimen. These results demonstrate that these aromatase inhibitors may be useful for the treatment of breast cancer.

INTRODUCTION

The extraglandular conversion of plasma androstenedione to estrone is an ongoing process throughout the life of a woman [1]. After menopause it becomes the principal source of estrogens [2]. The site of this aromatization is not known but it has been demonstrated that human fat [3,4], liver [5], and muscle [6] can synthesize these hormones from androgen. Since human mammary carcinoma is also capable of estrogen synthesis in vitro [7-10], a possible source in women with breast cancer is the tumor itself.

Because estrogen has been strongly implicated in promoting the growth of some mammary cancers, compounds which block the formation of these steroids could be useful in the treatment of this disease. It has

been demonstrated by Bellino et al. [11] that both 6α- and 6β-bromoandrostenedione inactivate the aromatase enzyme in human placenta. Δ^1-testololactone (Teslac®, Squibb and Lonesphic, Princeton, New Jersey), a drug which has been used with some success against breast cancer [12], has also been shown to inhibit human placental aromatase [13]. Barone et al. [14] found that treatment of breast cancer patients with this compound lowered the peripheral conversion of androstenedione to estrone and the level of plasma estrone.

This study reports data that 6α- and 6β-bromoandrostenedione, Δ^1-testololactone, and testololactone can inhibit the formation of estrogen from androstenedione by human mammary carcinoma in vitro.

MATERIALS AND METHODS

A. Steroids. [1β-^{3}H]-androstenedione was prepared from [1β, 2β-^{3}H]-androstenedione by drastic alkali treatment [15]. All other radioactive steroids were purchased from Amersham Searle, Arlington Heights, Illinois. Δ^1-Testololactone and testololactone were gifts from Dr. Albert Segaloff of the Alton Ochsner Medical Foundation and 6α- and 6β-bromoandrostenedione were given by Dr. Yoshio Osawa of the Buffalo Medical Foundation, Buffalo, New York.

B. Inhibition Studies. Tumor incubations were carried out as previously reported [8]. Briefly, tumor homogenates were incubated with [7α-^{3}H]-androstenedione (11Ci/mmol) and an NADPH generating system with and without inhibitors at 37°C. [4-^{14}C]-Estrone, -estradiol, and -estriol were then added to aid in identification and to determine recovery. The metabolites were extracted by solvent partition and the estrogen fraction was isolated on an ion-exchange column. The three estrogens were separated on thin layer chromatography and recrystallized to constant specific activity. For further proof of identity, the acetates were prepared, chromatographed and recrystallized.

C. Time study. To determine the time to be used for kinetic analysis, the aromatase assay of Bellino et al. [11] was used. Tumor homogenates containing 0.2 g tissue, 0.8 ml sucrose (0.25M), 0.2 ml Tris buffer (0.5M, pH 7.6), 7.2 mg glucose-6-phosphate, 1.2 mg NADP, and 5.6 units glucose-6-phosphate dehydrogenase were incubated with 400,000 DPM of [1β-^{3}H]-androstenedione (0.4μM) for 20, 40, 60, 120 and 180 minutes.

The reaction was stopped with 0.15 ml of 10% trichloroacetic acid. The mixture was applied to a column (0.5 x 10 cm) with a bottom layer of Amberlite XAD-2 resin (3 cm) and a top layer of granulated activated charcoal (3 cm) and eluted with 6 ml of water. The pmoles of estrogen synthesized are proportional to the amount of tritiated water eluted from the column.

RESULTS

Estrone and estradiol were the only estrogens identified as metabolites of androstenedione. Table 1 shows the data of two tumors incubated for 3 hours with 0.13 μM [7α-^{3}H]-androstenedione with and without 0.13 mM Δ^1-testololactone and testololactone. It is apparent that both tumors show appreciable inhibition when either of these compounds is added to the incubation mixture. Tumor #1 synthesized estrone and estradiol and the two inhibitors blocked the formation of both estrogens.

TABLE 1. Inhibition of Estrogen Synthesis from Androstenedione in Human Breast Tumors

Tumor	Inhibitor	Estrone	Estradiol
		(pmol/g tumor)	
1	None	0.8	1.6
	Δ^1-Testololactone	0.2	0.2
	Testololactone	0.4	0.4
2	None	0.6	--
	Δ^1-Testololactone	0.1	--
	Testololactone	0.2	--
4	None	0.4	--
	6α-Bromoandrostenedione	0.1	--
	6β-Bromoandrostenedione	0.1	--

The dose response effect of Δ^1-testololactone and testololactone, the time course of the reaction, and a kinetic analysis of the enzyme and its inhibitors were studied in a third tumor. Table 2 discloses

that the amount of estrogen formed decreases with increasing concentration of Δ^1-testololactone and testololactone (0.033, 0.065, 0.13 and 0.26 mM), although the inhibitory effects appear to level off at the highest dose. The amount of both estrone and estradiol synthesized is lowered by the presence of the inhibitors. The data show that Δ^1-testololactone is a better inhibitor than testololactone.

TABLE 2. Effect of Concentration of Inhibitor on Estrogen Synthesis from 0.13 μM Androstenedione in Human Breast Tumor

Inhibitor	Estrone (pmol/g tumor)	Estradiol (pmol/g tumor)	Inhibition (%)
None	9.2	1.3	
Δ^1-Testololactone			
0.033 mM	6.6	1.1	27
0.065 mM	3.6	0.7	59
0.13 mM	2.3	0.4	74
0.26 mM	1.6	0.3	82
Testololactone			
0.033 mM	6.8	0.9	27
0.065 mM	5.9	0.9	35
0.13 mM	3.7	0.8	57
0.26 mM	3.7	0.7	58

Study of the time course of the aromatase reaction (Figure 1) discloses that the amount of estrogen synthesized from [1β-^{3}H]-androstenedione increases linearly throughout the first 40 minutes of the reaction and then levels off. We therefore chose 30 minute incubations for the kinetic analysis studies.

Using the same breast tumor, incubation was carried out with concentrations of [7α-^{3}H]-androstenedione ranging from 0.05 to 0.8 μM. Since 90% of the estrogen formed by this tumor was estrone, the results from this experiment are reported in pmol of estrone. Figure 2, the reciprocal plot of the velocity of the reaction versus the substrate

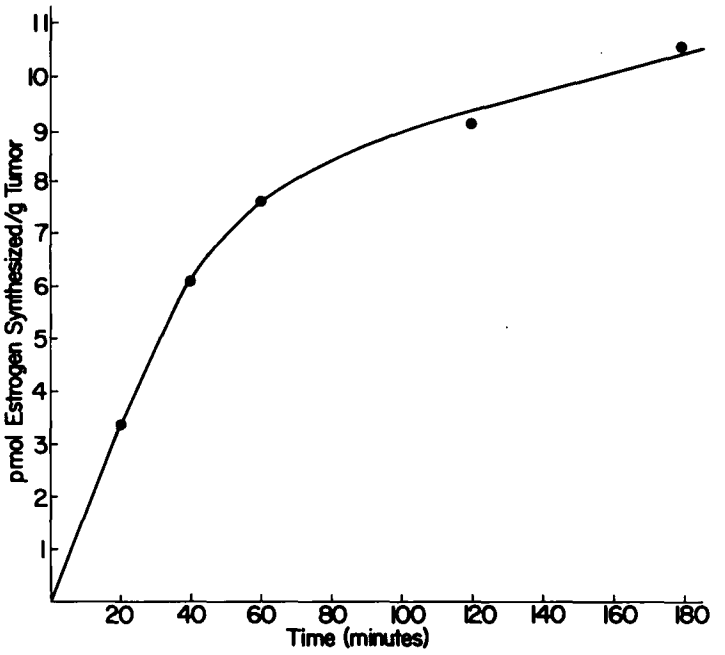

Figure 1. Time study - Incubation of Human Mammary Tumor with 0.4 μM androstenedione.

concentration, reveals an apparent K_m of the enzyme for the substrate of 0.08 μM and a V_{max} of 23 pmol of estrone synthesized per g tumor per hr. Δ^1-Testololactone (0.2 mM) and testololactone (0.2 mM) were added to the tumor incubations and the Lineweaver-Burk plot of the results is shown in Figure 3A. All three lines converge at the ordinate and this is indicative that both compounds are competitive inhibitors of the aromatase enzyme.

The effects of 6α- and 6β-bromoandrostenedione on the aromatase enzyme of this tumor were also studied. When added at the concentration of 2 μM to the incubations, these compounds both inhibit the aromatization of androstenedione. Again, the Lineweaver-Burk plot (Figure 3B)

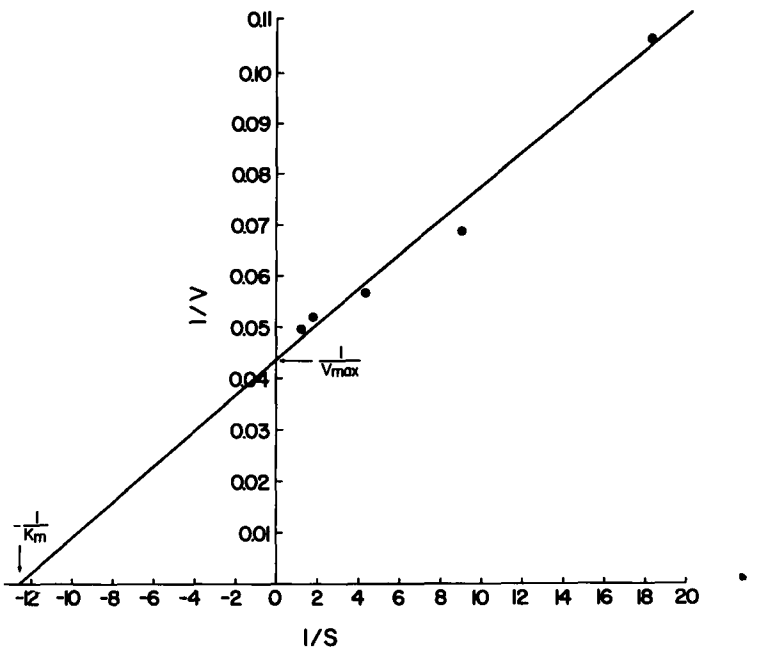

Figure 2. Kinetic Studies of Estrogen Synthesis from Androstenedione in Human Mammary Tumor. V = pmol estrone synthesized/g tumor/hr S = μM androstenedione

reveals that these compounds are competitive inhibitors of the enzyme. The 6α isomer was found to be more effective than the 6β compound.

A fourth tumor was incubated with 0.4 μM androstenedione with and without 2 μM concentration of bromominated androgens for 3 hours (Table 1). Since this tumor was done after the kinetic studies of the aromatase enzyme, the higher concentration of androstenedione was chosen for this and future studies. Again, both 6α- and 6β-bromoandrostenedione inhibit the formation of estrogen in the tumor.

DISCUSSION

Results from these experiments show that all four compounds tested are competitive inhibitors of the aromatase enzyme in human breast tumors.

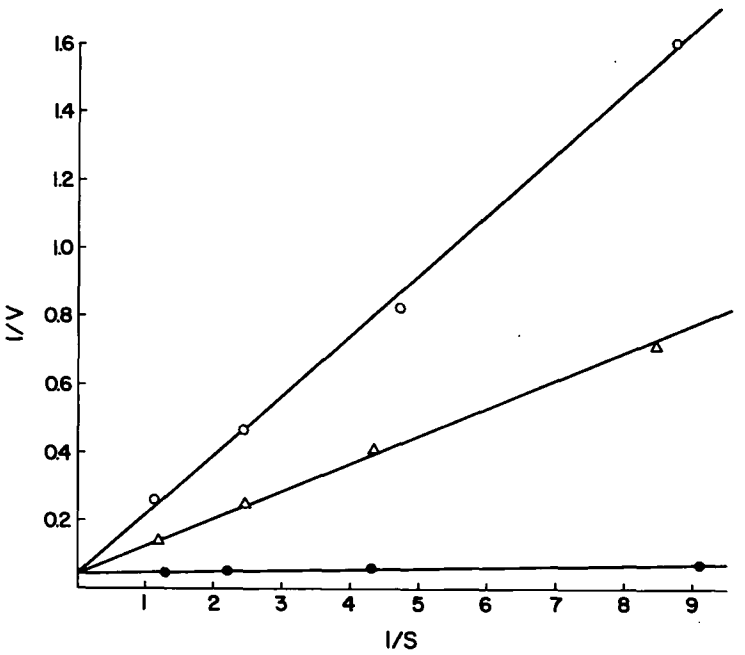

Figure 3A. Inhibition of estrogen synthesis from androstenedione in human mammary tumor by testololactone.
V = pmol estrone synthesized/g tumor/hr
S = μM androstenedione
• = No inhibitor
o = 0.2 mM Δ^1-testololactone
Δ = 0.2 mM testololactone

Δ^1-Testololactone was found to be more effective at reducing the level of estrogen than testololactone. 6α-Bromoandrostenedione was more potent than its 6β isomer and this parallels the results of Bellino et al. [11] using human placenta. Our data show that 6α-bromoandrostendione was the most effective aromatase inhibitor of the four compounds studied.

Although inhibition of peripheral metabolism of androstenedione by Δ^1-testololactone in breast cancer patients was reported by Barone et al. [14], our in vitro studies demonstrate that estrogen formation can be blocked directly at the tumor site. Furthermore, Barone et al. found

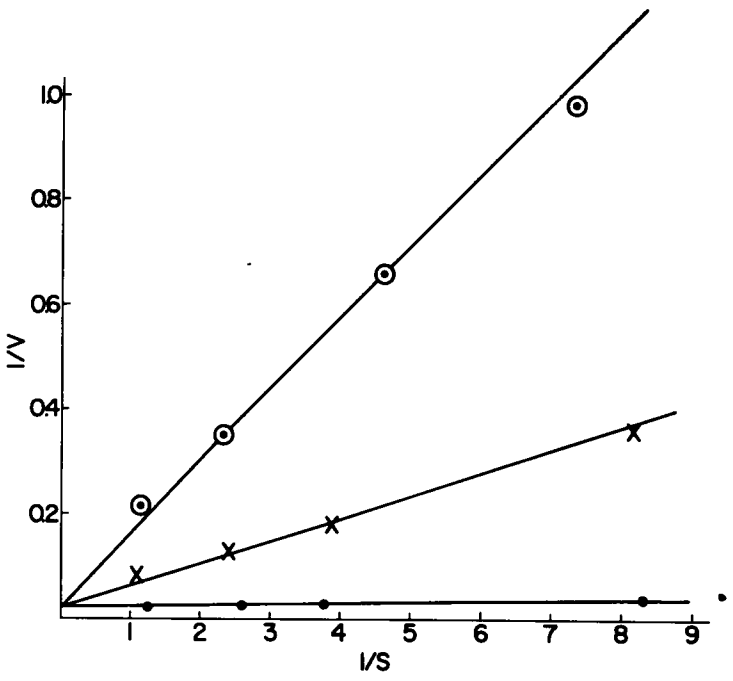

Figure 3B. Inhibition of estrogen synthesis from androstendione in human mammary tumor by bromoandrostenedione.
V = pmol estrone synthesized/g tumor/hr
S = μM androstenedione
• = no inhibitor
o = 2.0 μM 6α-bromoandrostenedione
X = 2.0 μM 6β-bromoandrostenedione

that only the level of plasma estrone was reduced in the presence of Δ^1-testololactone. We have shown that the concentration of estradiol was lowered as well.

Since breast tumor is well established as one site of peripheral formation of estrogens and we have shown that aromatase inhibitors can block this synthesis, such compounds could be of therapeutic value in breast cancer.

ACKNOWLEDGMENT

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The following trivial names have been used in the text:

Androstenedione: 4-androstene-3,17-dione
Estrone: 3-hydroxy-1,3,5(10)-estratrien-17-one
Estradiol: 1,3,5(10)-estratriene-3,17β-diol
Estriol: 1,3,5(10)-estratriene-3,16α,17β-triol
Δ^1-Testololactone: 17α-oxa-D-homo-1,4-androstadiene-3,17-dione
Testololactone: 17α-oxa-D-homo-4-androstene-3,17-dione