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Aromatase Inhibitors in Tumour Treatment

CLINICAL USE OF AROMATASE INHIBITORS IN HUMAN BREAST CARCINOMA

RICHARD J. SANTEN
Department of Medicine, Division of Endocrinology, The Milton S. Hershey Medical Center, The Pennsylvania State University, P.O. Box 850, Hershey, PA 17033, U.S.A.

Summary—The biological importance of aromatase rests in the concept that this is the rate-limiting enzyme involved in estrogen biosynthesis. Approx. one-third of human breast carcinomas depend upon estrogen for growth. Blockade of estrogen biosynthesis, then, provides an effective means of causing tumor regression in selected patients. The side effects and lack of specificity of the aromatase inhibitor, aminoglutethimide, provided the impetus toward development of nonsteroidal inhibitors of aromatase. Several compounds are currently being evaluated. Pyridoglutethimide is a derivative of aminoglutethimide which does not inhibit cholesterol side-chain cleavage and possesses no CNS sedative properties; the K_i for aromatase is 1100 nM, somewhat higher than for aminoglutethimide, 600 nM. CGS 16949A is a highly potent inhibitor of aromatase which is an imidazole derivative. This compound inhibits aromatase with a K_i of 0.19 nM whereas inhibition of C_{11}-hydroxylase activity occurs at 10^{-6} M. In clinical trials, this compound lowers plasma estrogen levels, blocks peripheral aromatization as documented by isotopic kinetic studies, and causes tumor regression. Phase III trials with this drug are now ongoing. Another agent, R76713, represents another highly potent and specific aromatase inhibitor with little toxicity in animal studies. The K_i for placental aromatase is 0.8 nM and this compound is approx. 500-fold more potent than aminoglutethimide. Phase I clinical studies in patients reveal a marked reduction in estrogen production. These compounds represent the most promising of a wide variety of agents currently being tested for their aromatase inhibitory properties.

INTRODUCTION

Human breast carcinomas are composed of two biological subtypes: those which are hormone-dependent and those which are hormone-independent. Estrogen is the primary steroidal mitogen for stimulation of growth of the hormone-dependent subtype. Several therapeutic strategies have evolved for treatment of hormone-dependent breast carcinoma [1]. Traditional methods include use of surgical ablative therapy such as hypophysectomy, adrenalectomy or oophorectomy. Pharmacological hormone additive therapy began with the introduction of diethylstilbestrol. High doses of progestin such as medroxyprogesterone acetate or megestrol acetate are now the predominant forms of additive therapy. Additional strategies focus upon the blockade of estrogen action with antiestrogens or the blockade of estrogen biosynthesis with either nonspecific inhibitors of steroidogenesis or specific inhibitors of aromatase. The current review focuses upon the current clinical status of aromatase inhibitors.

Aromatase inhibitors and antiestrogens both act by lowering the mitogenic effects of estrogen on breast tumor tissue. It is important to consider, then, the potential advantages of aromatase inhibitors versus the use of antiestrogens. One advantage of aromatase inhibitors is that they can specifically inhibit estrogen biosynthesis; currently available antiestrogens possess hormone agonist effects and specifically weak estrogenic effects [1]. Aromatase inhibitors, on the other hand, are devoid of such properties. The major advantage of aromatase inhibitors, however, is the lack of complete cross-resistance with antiestrogens. Studies reviewed from the literature indicated that of 263 women with advanced breast carcinoma initially treated with tamoxifen and later crossed over to aminoglutethimide (an aromatase inhibitor) and hydrocortisone, 31% of patients exhibited a secondary objective response to the aromatase inhibitor (Fig. 1) [2]. When the patients initially

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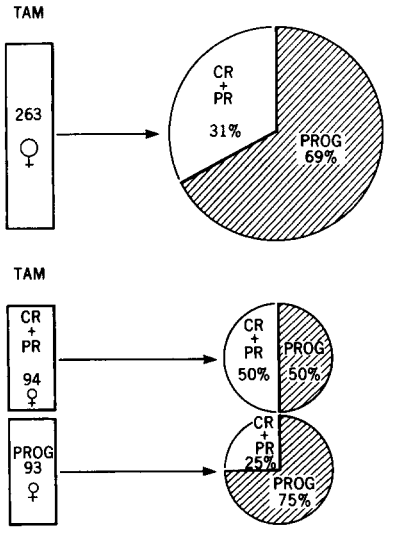

Fig. 1. Summary of several studies examining the response rates in patients initially treated with tamoxifen and then crossed over to aminoglutethimide plus hydrocortisone upon relapse. CR = complete objective tumor regression, PR = partial objective tumor regression, Prog = tumor progression. Top panel represents all patients. Bottom panel represents patients subdivided into those with CR and PR and those with tumor progression on exposure to tamoxifen.

treated with tamoxifen were sub-categorized, the lack of cross-resistance was even more evident. Specifically, of the 94 patients who experienced either complete or partial objective tumor regression with tamoxifen, 50% experienced a secondary objective tumor regression when crossed over to aminoglutethimide upon relapse. Of the 93 women initially progressing on tamoxifen, only 25% experienced a secondary objective response to aminoglutethimide.

SOURCES OF ESTROGEN

In order to fully understand the clinical use of aromatase inhibitors, it is necessary to review the sources of estrogen in women. Since currently available aromatase inhibitors are incapable of completely blocking ovarian estrogen biosynthesis in premenopausal women, the sources of estrogens in postmenopausal women only will be reviewed. The ovary contributes only a small fraction of the estrogen produced in postmenopausal women [3]. Similarly, the adrenal gland in postmenopausal women also does not directly secrete estradiol [4]. Notably, estrogens arise via the extraglandular conversion of androstenedione secreted by the adrenal into estrone [4]. This process occurs in fat tissue, muscle and liver. Through this pathway, postmenopausal women produce approx. 50–100 μg of estrone per day and more if obese [4]. A substantial fraction of estrone is converted to estradiol to produce circulating concentrations of 10–20 pg/ml. This level of estradiol should not be sufficient to occupy a biologically meaningful fraction of tumor estradiol receptors. However, the levels of estradiol in tumor tissue are an order of magnitude higher than in plasma and, thus, may be sufficient for an important level of receptor occupancy [5]. The mechanisms responsible for maintenance of high tissue estradiol concentrations are not clearly defined at present, but could potentially involve local production via either aromatase or sulfatase in the tumor or in tissues surrounding the tumor.

Several investigative groups identified aromatase activity in human breast tumors [6–8]. Compared to human placenta, absolute levels of activity are relatively low ranging from 5 to 100 pg/g tissue per h. Bradlow regarded this degree of activity to be too low for a meaningful level of estradiol to be synthesized locally [9]. Aromatase, however, could be localized to specific cell types such as that of fat cells, stroma, or certain epithelial tumor cells. If correct, biochemical measurement of total aromatase activity would under-estimate the levels of enzymatic activity present; e.g. in isolated epithelial tumor cells.

The biological importance of tumor aromatase rests in the concept that aromatase inhibitors could potentially block estradiol synthesis directly at the site of the tumor. Indirect support for this hypothesis comes from preliminary studies correlating tumor aromatase activity with clinical responses to aromatase inhibition [10]. Prospective trials of responses to aromatase inhibition in aromatase rich and aromatase poor tumors are now required to critically examine this issue.

Our studies have evaluated the potential importance of tumor concentrations of sulfatase, aromatase, and 17β-hydroxysteroid dehydrogenase [6]. We have measured the levels of aromatase in human breast tumor homogenates and compared these with levels in MCF-7 cells in culture. Since MCF-7 cell lines differ from laboratory to laboratory, we have concentrated our studies on a cell line characterized by Dr Robert Brueggemeir. As shown in Fig. 2, aromatase activity was similar in human tumor homogenates as well as in MCF-7 cells. However, the levels of sulfatase and 17β-hydroxysteroid dehydrogenase were 5–6 orders of magnitude higher in human tumors than were the levels of aromatase.

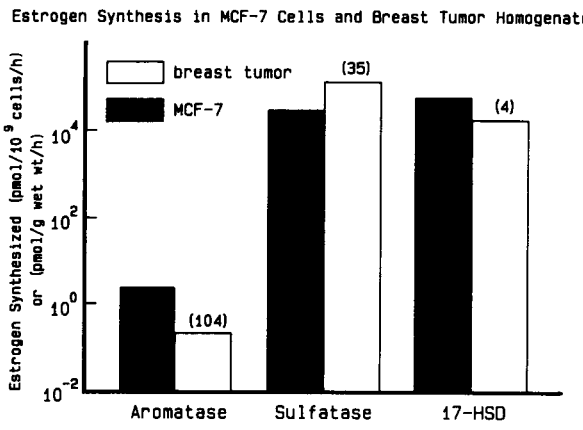

Fig. 2. Comparison of enzyme levels in human breast tumors and in MCF-7 cells in culture. Data summarized from Refs [6, 11].

Even though the enzymes to produce estradiol are present in human breast tumors, the biological significance of these pathways is currently unsubstantiated. In order to provide further data regarding this, we have examined the effects of estrone sulfate, estrone, and estradiol on the growth of MCF-7 cells in culture [11]. We demonstrated a dose–response stimulation of cell number using concentrations of 1–1000 nM of estrone sulfate. Significant stimulation was observed with 100–1000 nM concentrations of the sulfated estrone. This could be compared with a maximal stimulatory dose of estradiol of 10^{-9} M. The effects of estrone sulfate were mediated through the estrogen receptor since the pure antiestrogen ICI-164384 markedly inhibited the number of cells present after incubation for 6 days with 10^{-6} M estrone sulfate. As a further parameter reflecting stimulation of mitogenesis, the S-phase was significantly stimulated by estrone sulfate as well and this effect could be inhibited by use of the pure antiestrogen 164384. Similar dose–response studies were conducted with free estrone. Concentrations of 0.1–100 nM stimulated the growth of MCF-7 cells. In general, one log higher concentrations of estrone were necessary to stimulate cell growth when compared with free estradiol. In contrast to the effects of estrone sulfate and estrone, we were unable to demonstrate stimulatory effects of androstenedione on MCF-7 cell growth. It was considered that the lack of stimulation might reflect the androgenic properties of the 300 nM concentration of androstenedione used in these experiments. However, coculturing with 2-hydroxyflutamide, a specific antiandrogen, elicited no increase in cell growth in flasks containing androstenedione.

These *in vitro* results led to the hypothesis that estrone sulfate can stimulate epithelial breast cancer cells through an *autocrine* mechanism. These cells contain sufficient sulfatase and 17β-hydroxysteroid dehydrogenase to allow estrone sulfate to be converted into estradiol. Estradiol then binds to the nucleus of these cells and initiates a cascade of events resulting in cell proliferation. In support of this hypothesis, the major tritiated steroid present in the nucleus after incubation with tritiated estrone sulfate was estradiol [11]. The concentrations of estradiol in the nucleus after estrone sulfate incubation were similar to those found in cells exposed to the 10^{-9} M tritiated estradiol.

The role of aromatase in human breast cancer cells *in vitro* is as yet unclear. Based upon a variety of data, we hypothesize that the stromal or fat cells in the tumor contain aromatase and that androstenedione may be converted to estrone and then estradiol in these tissues [12, 13]. Through a *paracrine* mechanism, the estrone produced in the stromal cells could then bind to receptors within the epithelial cells. As an additional pathway, estradiol, circulating in the plasma of postmenopausal patients, might also enter breast cancer epithelial cells and result in their stimulation through an *endocrine* mechanism. Further studies are ongoing to specifically evaluate each of these pathways.

HISTORICAL DEVELOPMENT OF AROMATASE INHIBITORS

Aminoglutethimide was initially recognized to be an inhibitor of cytochrome *P*450 mediated steroid hydroxylations and partially of those involving the cholesterol side chain cleavage enzyme [14]. The first clinical use of aminog-

lutethimide for breast cancer attempted to produce a "medical adrenalectomy" by blocking cholesterol side chain cleavage [14–16]. Replacement glucocorticoid was added to compensate for the inhibition of cortisol biosynthesis.

While these studies were ongoing, however, Thompson and Siiteri [17] demonstrated the potent aromatase inhibitory properties of aminoglutethimide *in vitro*. This followed logically from their demonstration that each of the three hydroxylations catalyzed by aromatase requires the participation of a cytochrome *P*450 step. Direct isotopic kinetic studies in patients then confirmed the potency of aminoglutethimide as an aromatase inhibitor *in vivo* [18]. A dose of 1000 mg of aminoglutethimide daily produced 95–98% inhibition of aromatase in postmenopausal women with breast cancer. These studies involved the administration of tritiated androstenedione and the measurement of the aromatized product, tritiated estrone. C-14 estrone was administered intravenously at the same time in order to allow correction for the metabolic transformation of estrone into other products. Isolation of radiochemically pure estrone and determination of tritium to C-14 ratios in either plasma or urine provided the endpoint of this ρ-value determination.

Studies of the ability of aminoglutethimide to block human breast tumor aromatase were indirect. Breast tumor homogenates were incubated with 1–100 μM aminoglutethimide [19]. Eighty per cent inhibition of aromatase could be observed with the highest concentration of aminoglutethimide. Since plasma concentrations of this drug reached 30 μM, it could be extrapolated that approx. 70% inhibition of aromatase would occur during aminoglutethimide/hydrocortisone administration. A final endpoint of assessment of aromatase inhibition was the measurement of plasma and urinary estrogens. It was assumed that suppression of estrogens to the same level as observed after surgical adrenalectomy in postmenopausal women would reflect nearly complete estrogen inhibition. No significant differences in the levels of plasma and urinary estrone and estradiol or plasma estrone sulfate were observed between patients receiving aminoglutethimide/hydrocortisone and those having undergone bilateral surgical adrenalectomy [20].

Based upon these studies, a standard regimen of 250 mg of aminoglutethimide four times daily and 20 mg of hydrocortisone twice daily was developed to uniformly inhibit aromatization in postmenopausal patients with breast cancer. Pilot trials and comparisons with tamoxifen and medroxyprogesterone acetate indicated that this approach was as effective as other endocrine agents in producing objective tumor regressions in patients with advanced breast cancer [1]. However, standard doses of aminoglutethimide inhibit multiple cytochrome *P*450-mediated steroid-hydroxylations such as cholesterol side chain cleavage, C11-hydroxylase, and 18-hydroxylase [1]. In addition, the standard 1000 mg doses of aminoglutethimide were associated with side effects such as lethargy, skin rash, ataxia, and orthostatic dizziness [1]. For this reason, major emphasis has been placed upon development of more potent and more specific inhibitors of aromatase.

DEVELOPMENT OF SPECIFIC AROMATASE INHIBITORS

Enhanced potency and specificity as well as reduced side effects are the primary goals of development of new aromatase inhibitors. A wide variety of compounds are under study at present [21]. A convenient classification divides inhibitors into the mechanism based or "suicide" inhibitors and those of a competitive type [1]. "Suicide" inhibitors initially compete with the natural substrate (i.e. androstenedione and testosterone) for binding to the active site of the enzyme. The enzyme then specifically acts upon the inhibitor to yield reactive species which can form covalent bonds at or near the active site of the enzyme. Through this mechanism, the enzyme is irreversibly inactivated. A potential advantage of the mechanism-based inhibitors is their ability to more completely destroy enzyme activity.

Competitive inhibitors bind reversibly to the active site of the enzyme and prevent product formation only as long as the inhibitor occupies the catalytic site. Competitive inhibitors consist of both steroidal and nonsteroidal compounds. Advantages of the steroidal inhibitors are their intrinsic ability to inhibit the aromatase enzyme specifically. Disadvantages of the steroidal inhibitors are the potential for inducing agonist or antagonist effects, particularly on estrogen, glucocorticoid, androgen, or progesterone receptors. In addition, the steroidal inhibitors are metabolized widely to other compounds which can interfere with the measurement by radioimmunoassay of estrogens in plasma or in urine.

In general, nonsteroidal inhibitors are more likely than are steroidal compounds to lack specificity since they have a potential for blocking several cytochrome *P*450 mediated steroid hydroxylations. On the other hand, nonsteroidal inhibitors are unlikely to possess agonist or antagonistic properties for various steroids and are more likely to be orally absorbed. At the present time, several nonsteroidal aromatase inhibitors are being developed including pyridoglutethimide, R76713, CGS 16949A, and CGS 20267 (Table 1). From preliminary data, the oral doses of these compounds include 200–1600 mg for pyridoglutethimide, 1–5 mg for R76713, 0.6–4 mg for CGS 16949A, and 0.2–30 mg for CGS 20267.

PHASE I AND II STUDIES WITH CGS 16949A

As a background for understanding the development of aromatase inhibitors, the studies conducted in our laboratory with CGS 16949A will be reviewed. A phase I study was conducted [22] which sought to determine the toxicity of CGS 16949A, the efficacy of estrogen suppression, the specificity of aromatase inhibition and the pharmacokinetics of this compound. In general, no significant toxicity was observed. The plasma half-life of the drug in patients receiving standard doses was in the range of 10–11 h.

The rationale of our phase I study was that all nonsteroidal aromatase inhibitors can block other cytochrome *P*450-mediated steroid hydroxylations as long as the dose is sufficiently high. Consequently, we conducted a dose ranging study which examined the effects of 0.6–16 mg of CGS 16949A daily in 12 postmenopausal women with metastatic breast cancer [22]. Inhibition of aromatase, as evidenced by a fall in estrone and estradiol levels without a rise in androstenedione occurred at a dose of 0.6 mg daily. Maximal inhibition of estrogen levels was observed at doses between 2 and 4 mg daily. The effect of CGS 16949A at this dosage appeared to be relatively specific. However, when 8–16 mg daily were administered, increments of 17α-hydroxyprogesterone, androstenedione, and testosterone occurred suggesting blockade of the C-11 or C-21 hydroxylase steps in addition. At the 16 mg dose, ACTH levels increased even though basal cortisol levels were unchanged, suggesting an effect on cortisol biosynthesis [22]. This effect was confirmed by the demonstration of blunted cortisol responses to exogenous ACTH (cortrosyn) at this dosage. Lamberts *et al.* [23] recently demonstrated similar effects on isolated adrenal cells *in vitro* and attributed this to the C-11 hydroxylase inhibitory properties of the drug.

An effect on aldosterone biosynthesis was also observed at high doses since basal levels declined during the administration of 8 and 16 mg daily and ACTH stimulation of aldosterone was completely inhibited [24]. Further examination of the aldosterone pathway revealed that CGS 16949A blocks the corticosterone methyloxidase type II step and increases the ratio of plasma 18 hydroxycorticosterone to aldosterone as well as urinary tetrahedro-compound-A to tetrahedro-aldosterone [24]. Studies in rats by Bhatnager [25] also indicated that aldosterone is inhibited through the action of this compound on the 11β-hydroxylase step involved in aldosterone biosynthesis.

Results from the initial dose seeking studies led to a phase II study which compared doses of 0.6 mg 3 times daily, 1 mg twice daily, and 2 mg twice daily [26]. Maximal suppression of plasma and urinary estrogens occurred at a dosage of 1 mg twice daily and minimal effects on cortisol secretion were observed. Basal cortisol and

Table 1. Partial list of aromatase inhibitors

Type of inhibition	Type of compound	Name of compound	K_i	K_{inact}
Mechanism-based	Steroid	1,4,6-Androstatriene-3,17-dione		$1.1 \times 10^{-3} S^{-1}$
	Steroid	4-OH-Androstenedione		$4.5 \times 10^{-3} S^{-1}$
	Steroid	4-Androstene-3,6,17-trione		$4.03 \times 10^{-3} S^{-1}$
	Steroid	Testolactone		$5.5 \times 10^{-4} S^{-1}$
	Steroid	10β-Propargylestr-4-ene-3,17-dione		$1.11 \times 10^{-3} S^{-1}$
	Steroid	7α(4′-Amino)phenylthio,1,4-androstadiene-3,17-dione		$8.4 \times 10^{-3} S^{-1}$
	Steroid	1,-Methyl-androsta-1,4-diene,3,17-dione		$1.8 \times 10^{-4} S^{-1}$
Competitive	Steroid	6α-Bromo-androstenedione	3.4 nM	
	Steroid	7α(4′-Amino)phenylthio-4-androstene-3,17-dione	18 nM	
	Nonsteroid	Aminoglutethimide	540 nM	
	Nonsteroid	Pyridoglutethimide	1100 nM	
	Imidazole	CGS 16949A	0.19 nM	
	Triazole	R76713	0.70 nM	
	Imidazole	Econazole	0.06 μM[a]	

[a] IC_{50}. Table reproduced from Ref. [1]; specific references to compounds reproduced in that reference.

ACTH levels were unaffected and cortisol levels increased to more than 20 μg/100 ml after exogenous cortisone administration in all patients. Basal levels of aldosterone also remained stable upon administration of all three drug dosages. No change in urinary or plasma sodium or potassium was observed. There were no changes in standing blood pressure to suggest a clinical state of aldosterone deficiency. However, cortrosyn stimulated aldosterone levels were significantly blunted at either of the three doses.

We further examined the effect of CGS 16949A on C-11 hydroxylase in these patients. 11-desoxycortisol levels were obtained under basal conditions prior to and at 30 and 60 min after 0.25 mg of cortrosyn. This adrenal stimulatory protocol was then repeated after patients had been receiving CGS 16949A for 8 and 12 weeks. Levels of 11-desoxycortisol increased in response to CGS 16949A both under basal and ACTH-stimulated conditions. These increments were minimal with peak levels after ACTH reaching approximately 0.8 μg/dl. Average responses in 11-desoxycortisol after 3.0 g of metyrapone were 6.9 ± 0.54 μg/ml. Based on these observations, we conclude that CGS 16949A is a weak inhibitor of 11β-hydroxylase. This effect is much less than that observed after a standard test dose of metyrapone.

The antitumor activity of CGS 16949A in patients is not as yet precisely defined as limited clinical data are available. In our phase I study, two of 12 heavily pretreated patients [27] experienced objective tumor regression. In the phase II trial of 54 patients, 3 complete and 8 partial objective tumor regressions were observed. In 25 patients, there was no change in measurable tumor dimensions, and in 18 patients tumor progression was observed [2]. The mean duration of responses in patients who experienced complete or partial objective tumor regressions was 14 months (7–18 + months). The sites of responses were predominantly soft tissue and bone.

OTHER EMERGING AROMATASE INHIBITORS

Nonsteroidal agents lacking C-11 hydroxylase or aldosterone blocking properties are being developed (Table 1). One of these agents is a triazole R76713 [28]. The K_i of this compound for aromatase is 0.80 nM in human placental microsomes. This is to be compared with the K_i of 0.17 nM for CGS 16949A. The enantiometers of R76713 are currently being evaluated. These are designated R83839 and R83842 [29]. The R83842 possesses the majority of the aromatase inhibitory potency of the racemic mixture. Preliminary studies with this agent reveal its ability to markedly inhibit estradiol production, to cause tumor regression in DMBA and NMU rat mammary tumors and its ability to inhibit ovarian estrogen production in the rat [30]. Further clinical studies are forthcoming with the enantiomers of this compound.

An additional triazole CGS20267 is also under clinical study [2]. This agent appears to be effective at a dosage as low as 200 μg/day given orally to men. In animal studies, no inhibitory activity is present with the cholesterol side chain cleavage enzyme, C-11-hydroxylase or C1720 lyase.

Practical means of assessing aromatase inhibition

Future studies will require comparison of the degree of estrogen suppression with various aromatase inhibitors. Use of highly sensitive and specific plasma estradiol assays provides one means of evaluating these compounds. Another method involves use of the isotopic kinetic techniques to determine total body aromatase rates. In our studies with CGS 16949A, basal ρ values are $2.56 \pm 0.58\%$ ($n = 8$). Patients receiving CGS 16949A in doses ranging from 1.8 to 4 mg daily have levels of aromatization of $0.35 \pm 0.05\%$. This represents a statistically significant ($P < 0.01$) suppression of 86%. Use of more potent aromatase inhibitors should be likely to induce complete inhibition of aromatization as detection by this methodology. The significance of suppression of aromatization below 85%, however, is as yet not established.

Perspectives for aromatase inhibitors

The availability of additional aromatase inhibitors should have several advantages. These include (1) the provision for selective methods of inhibiting estrogen biosynthesis, (2) the availability of agents with no estrogen agonistic properties. Nearly all of the currently available antiestrogens produce estrogen agonistic effects under certain clinical circumstances, (3) newly available aromatase inhibitors should be effective as second-line hormonal agents after tamoxifen for breast cancer. If these agents are devoid of side-effects and significant toxicity, they would be preferable to use of the progestins in either moderate or high doses, (4) availability of new aromatase inhibitors should widen the therapeutic options for patients with breast cancer.

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