

Activity of 17 β -(N-Alkyl/arylformamido) and 17 β -[(N-Alkyl/aryl) alkyl/arylamido]-4-methyl-4-aza-5 α -androstan-3-ones as 5 α -Reductase Inhibitors in the Hamster Flank Organ and Ear¹

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Skin disorders such as acne, seborrhea, hirsutism, and androgenic alopecia are secondary to excess local androgenic activity. Because the most potent androgen, dihydrotestosterone, is formed from testosterone by the action of 5 α -reductase, the inhibition of 5 α -reductase is a logical approach to interfere with androgenic action in the skin. In this study, we have investigated the inhibitory effect of a series of 17 β -(N-alkyl/arylformamido)- and 17 β -[(N-alkyl/aryl)alkyl/arylamido]-4-methyl-4-aza-5 α -androstan-3-one derivatives as 5 α -reductase inhibitors following their topical application on the flank organs and ears of Golden Syrian hamsters. The parameters measured were mainly the size of the underlying sebaceous glands and 5 α -reductase activity in the flank organs and ears. We found that 17 β -(N-amyloformamido)-4-methyl-4-aza-5 α -androstan-3-one (EM-401), 17 β -(N-hexylformamido)-4-methyl-4-aza-5 α -androstan-3-one (EM-402), and 17 β -(N-heptylformamido)-4-methyl-4-aza-5 α -androstan-3-one (EM-540) are potent inhibitors of 5 α -reductase activity. EM-

402 decreases the size of treated flank organs by 22%, 31%, and 32% ($p < 0.01$ for all) after topical application at the doses of 30, 100, and 300 μ g, respectively, twice daily for 4 wk. EM-402 also reduced the size of underlying sebaceous glands by 38%, 42%, and 59% of intact control values at the same doses. Comparable results were observed on the size of the sebaceous glands of the ears. In addition, we have observed a concentration-dependent 47%–80% ($p < 0.01$) and 46%–80% ($p < 0.01$) inhibition of 5 α -reductase activity in the right flank organs and ears, respectively, using topical EM-402. EM-402 had no significant effect on the same parameters in the left contralateral flank organs or ears. In addition, EM-402 had no effect on prostatic and seminal vesicle weights whereas EM-401 and EM-540 showed some systemic effects. These data illustrate that EM-402 applied topically, at the concentrations used, exerts a potent local anti-androgenic effect without any systemic action in the hamster. **Key words:** acne/androgens/costovertebral organ/sebaceous glands. *J Invest Dermatol* 111:273–278, 1998

The relationship between androgenic hormones and skin physiology has been an attractive area of research for endocrinologists and dermatologists for several decades (Pochi and Straus, 1964; Cunliffe and Shuster, 1969a). It is now well recognized that acne, hirsutism, and male pattern baldness are all androgen-related disorders (Darley *et al*, 1982; Barth, 1988). Moreover, patients suffering from acne usually present seborrhea or an excess of serum production (Cunliffe and Shuster, 1969b). In addition to excess production of androgens by the ovaries and adrenals (through DHEA), a hyperandrogenic state can also result from an increase in the 5 α -reduction of testosterone in the skin (Barth, 1988). In fact, testosterone, the main androgen of testicular and ovarian origins, is converted into dihydrotestosterone (DHT) in the target cell by 5 α -reductase (Labrie, 1991).

Blockade of androgenic action can be achieved through several mechanisms, e.g., by inhibiting the conversion of testosterone to DHT

with 5 α -reductase inhibitors, whereas the action of both testosterone and DHT may be efficiently prevented from binding to the androgen receptor by competition with a receptor-binding compound possessing no androgenic activity, i.e., a pure anti-androgen (Voigt and Hsia, 1973; Neumann and Steinback, 1991; Plewig and Luderschmidt, 1991). In fact, a series of steroidal and nonsteroidal compounds have been tested in animals or humans (Lutsky *et al*, 1975; La Vecchia *et al*, 1984; Luderschmidt *et al*, 1984; Weissmann *et al*, 1985; Bouton *et al*, 1986; Brooks *et al*, 1991; Lookingbill *et al*, 1992; Cusan *et al*, 1993, 1994; Matias and Gaillard, 1995; Labrie *et al*, 1996). Systemically administered anti-androgens, such as 17 α -methyl-B-nortestosterone and flutamide as well as the partial androgen antagonists spironolactone and cyproterone acetate, have been found to improve acne, hirsutism, and androgenic alopecia in women (Zarate *et al*, 1966; Lookingbill *et al*, 1992; Cusan *et al*, 1993, 1994); however, these applications are limited because of the systemic action of these anti-androgens. The pure anti-androgenic compound flutamide has been found to be more potent than the mixed androgenic/anti-androgenic compound spironolactone (Cusan *et al*, 1993). It would be of great therapeutic value to make available drugs that have pure local anti-androgenic activity without causing systemic side-effects.

Two types of human 5 α -reductase, chronologically identified as type I (Andersson and Russel, 1990; Harris *et al*, 1992) and type II (Andersson *et al*, 1991; Labrie *et al*, 1992) 5 α -reductase have been isolated from human prostatic cDNA libraries, and the structure of

Manuscript received October 12, 1997; revised February 24, 1998; accepted for publication April 14, 1998.

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¹This research has been supported by Endorecherche that is seeking patent protection for the new compounds. F. Labrie is President of Endorecherche.

the two isoenzymes has been elucidated. Type I 5 α -reductase is predominately expressed in the skin (Andersson and Russel, 1990; Harris *et al*, 1992; Luu-The *et al*, 1994), whereas the type II enzyme is responsible for male pseudohermaphroditism when defect.

We have previously reported that a series of 17 β -(N-alkyl/arylformamido)- and 17 β -[(N-alkyl/aryl)alkyl/arylamido]-4-methyl-4-aza-5 α -androstan-3-one derivatives strongly inhibit human type I 5 α -reductase activity and show low potency on human type II 5 α -reductase in transfected cells *in vitro* (Li *et al*, 1995). Because the androgen sensitivity of the Syrian hamster flank organs and ears have made this animal a useful model for the study of such compounds (Hamilton and Montagna, 1950; Plewig and Luderschmidt, 1982; Vermorken *et al*, 1982; Matias and Gaillard, 1995), we have evaluated the anti-androgenic activity of these compounds on Syrian hamster flank organs and ears after a 4 wk topical administration.

MATERIALS AND METHODS

Animals and experimental procedure Male Syrian hamsters \approx 8–10 wk old and weighing 110–120 g were purchased from Charles-River Laboratories (St. Constant, Québec, Canada) and housed 4–5 per cage in a light (14 h light per d, lights on at 07:15) and temperature (21 \pm 3°C) controlled environment. The animals were housed in plastic boxes on sawdust and received hamster chow (Agway Country Food, Syracuse, NY) and tap water *ad libitum*. At the beginning of the experiment, the ear was marked for identification purposes and the area of the flank organ was shaved with electric clippers. The appropriate doses of the indicated compounds were dissolved in ethanol-propylene glycol (50:50, vol/vol) and administered in 10 μ l on the right side of the hamster flank organs as well as the ears. Meanwhile, the left side of the flank organs and ears of the animals of the control and treated groups received the vehicle only. The three experiments were performed as follows:

Experiment 1 Intact hamsters (four per group) were treated with EM-423, 347, 401, 402, 422, 435, 336, 337, 436, 540, 541 at a dose of 50 μ g, twice daily, on the right flank organ.

Experiment 2 Intact hamsters (four per group) were treated with EM-424, 486, 497, 494, 493, 498, 503, 580, 568, 606, 567, 682 at a dose of 100 μ g, twice daily, on the right flank organ.

Experiment 3 Intact hamsters (12 per group) were treated with EM-401, 402, and 540 at doses of 30, 100, and 300 μ g, twice daily, on the right flank organ and ear.

The 5 α -reductase inhibitors were administered twice daily for 28 consecutive days. The length and width of the darkly pigmented oval spot outlining each flank organ were carefully measured with Vernier calipers (Fisher Scientific, Pittsburgh, PA). Flank organ area was then calculated according to the following formula: area of ellipse = $\pi(L/2 \times W/2) = 3.14 \times (L \times W)/4$. The animals were killed by decapitation on the morning following the last dose. The flank organs and ears were then removed quickly and fixed for histologic examination or frozen in dry ice, and stored at -80°C for enzymatic assays. The ventral prostate as well as the seminal vesicles were removed, dissected, freed from fat and connective tissue, and rapidly weighed.

Measurement of sebaceous gland size The flank organs and ears were fixed in 4% paraformaldehyde for 48 h and then rinsed in 15% sucrose phosphate buffer for 24 h at 4°C. The flank organs were then cut in the middle in the rostral to caudal direction and the ears were cut between the first and second cartilage ridges. The tissues were then embedded in tissue-tek (Miles, Diagnostic Division, Elkhart, IN) and cut with a cryostat in 8 μ m thick sections before staining with hematoxylin and eosin. The surface areas of the sebaceous glands of flank organs were estimated with a computer-assisted Image-Pro Plus program (Media Cybernetics, Silerspring, MD), and the surface areas of the sebaceous glands of the inner surface of the ears were estimated directly under optical microscopy using a grid. The surface area was counted as square units occupied by sebaceous glands in each section area. Six consecutive sections of each sample were analyzed for both flank organ and ear. The total number of arbitrary units was counted and the mean of six slides from each sample was calculated. Only the glands of the inner surface of the ear were measured. The results were calculated in units and expressed as a percentage of the control group that was taken as 100%.

Measurement of 5 α -reductase activity The enzymatic assay was performed as described previously (Martel *et al*, 1994). In brief, 100 μ l aliquots of the 1000 \times g supernatant of hamster flank organs and ears were incubated for 3 h at 37°C in a total volume of 0.5 ml phosphate buffer (12.5 mM KH₂PO₄, 1 mM ethylenediamine tetraacetic acid, pH 7.5) containing 0.5 μ M [4-¹⁴C]-

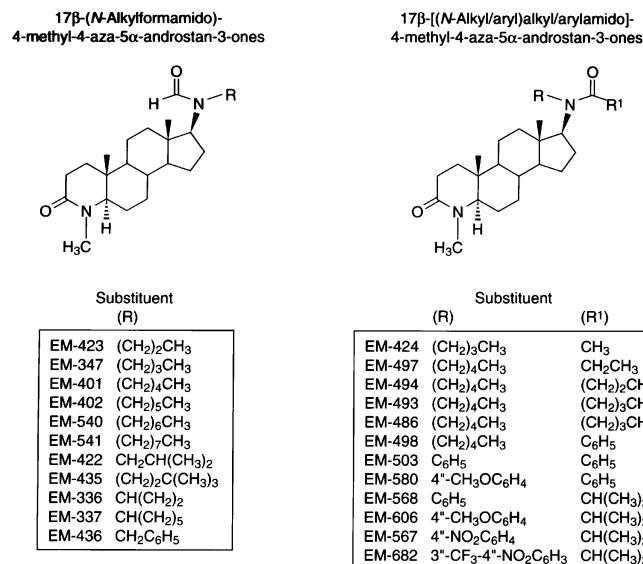


Figure 1. Structure of novel compounds that act as 5 α -reductase inhibitors. 17 β -(N-alkylformamido)-4-methyl-4-aza-5 α -androstan-3-ones: EM-423, EM-347, EM-401, EM-402, EM-540, EM-541, EM-422, EM-435, EM-336, EM-337, and EM-436. 17 β -[(N-alkyl/aryl)alkyl/arylamido]-4-methyl-4-aza-5 α -androstan-3-ones: EM-424, EM-497, EM-494, EM-493, EM-486, EM-498, EM-503, EM-580, EM-568, EM-606, EM-567, and EM-682.

testosterone (S.A., 51.4 mCi per mmol) and the cofactor NADPH. Labeled radioactivity was purchased from New England Nuclear/Dupont (Markham, Canada) and purified by thin-layer chromatography before use. The enzymatic reaction was stopped by chilling the incubation mixture in an ice-water slurry and adding 3 ml of diethyl ether. The components were then mixed and frozen in a dry ice-ethanol bath. The organic phase was kept while the remaining frozen aqueous fraction was re-extracted once with ether. The organic phases were then pooled and evaporated to dryness under a nitrogen stream. All components were then separated on thin-layer chromatography (60 F₂₅₄ silica gel, Merck, Darmstadt, Germany) using toluene:acetone (4:1, vol/vol) as solvent before autoradiography of the plates for 48 h. The metabolites revealed by autoradiography were identified by comparison with standard labeled steroids. The thin-layer chromatography areas corresponding to testosterone, DHT, and the DHT metabolites androstane-3 α ,17 β -diol and androstane-3 β ,17 β -diol were scraped and transferred into vials containing 0.5 ml ethanol and 10 ml scintillation liquid. The radioactivity was then measured in a liquid scintillation counter. Results are expressed as means \pm SEM in pM product formed per mg protein per min.

Statistical analysis Statistical significance was measured according to the multiple-range test of Duncan-Kramer (Kramer, 1956). Data are expressed as means \pm SEM.

RESULTS

Novel 17 β -(N-alkyl/aryl formamido)-4-methyl-4-aza-5 α -androstan-3-ones show topical anti-androgenic activity A series of novel 17 β -(N-alkyl/arylformamido)-4-methyl-4-aza-5 α -androstan-3-ones (Fig 1) were tested by application on hamster flank organs. The compounds were administered topically on the right flank organs of Golden Syrian hamsters at a dose of 50 μ g, twice daily, for 4 wk, whereas the control animals and the left side of the animals of the treated groups received the vehicle only. As can be seen in Table I, the surface area of the right side flank organs (treated side) of hamsters treated with EM-347, EM-402, EM-540, EM-337, EM-436, and EM-435 decreased by 27% ($p < 0.01$), 26% ($p < 0.01$), 24% ($p < 0.01$), 17% ($p < 0.05$), 21% ($p < 0.05$), and 25% ($p < 0.01$), respectively, whereas the surface area of the left side flank organs (the side treated with vehicle only) was unaffected. On the other hand, compounds EM-401 and EM-541 demonstrated systemic activity because they reduced the surface area of both side flank organs by 24% ($p < 0.01$), 26% ($p < 0.01$), 18% ($p < 0.01$), and 25% ($p < 0.01$), respectively. Treatment with EM-423, EM-422, and EM-336 did not affect flank organ size. It can be seen in the same table that the weights of the

Table I. Inhibitory effects of 17 β -(N-alkylformanido)-4-methyl-4-aza-5 α -androstane-3-one derivatives on the size of flank organs as well as ventral prostate and seminal vesicle weight. Compounds were applied on the right flank organ^a

EM (50 μ g, BID)	Flank organ surface area (mm ²)		Ventral prostate weight (mg)	Seminal vesicle weight (mg)
	Left side	Right side		
Control	33.5 \pm 2.1	33.4 \pm 1.3	53.9 \pm 4.1	228.4 \pm 6.4
423	34.9 \pm 3.1	34.8 \pm 1.3	55.3 \pm 4.0	242.7 \pm 6.9
347	32.0 \pm 1.3	24.6 \pm 1.0**	55.0 \pm 3.2	208.0 \pm 12.1
401	25.3 \pm 1.8**	24.6 \pm 2.0**	54.1 \pm 1.6	249.5 \pm 17.0
402	32.1 \pm 0.9	24.6 \pm 1.2**	50.0 \pm 0.9	226.5 \pm 25.5
540	32.9 \pm 0.8	25.1 \pm 0.7**	50.7 \pm 3.0	248.9 \pm 6.4
541	27.5 \pm 1.4*	25.0 \pm 0.5**	55.5 \pm 1.0	256.0 \pm 3.6
422	30.4 \pm 2.2	28.2 \pm 1.8	58.2 \pm 2.8	227.3 \pm 22.3
435	29.3 \pm 0.9	25.2 \pm 1.3**	59.0 \pm 6.0	223.9 \pm 20.7
336	32.3 \pm 0.6	33.2 \pm 1.1	49.3 \pm 4.5	212.1 \pm 10.2
337	32.4 \pm 1.2	27.7 \pm 0.8*	55.4 \pm 2.1	221.7 \pm 23.8
436	36.1 \pm 1.8	26.3 \pm 1.7*	58.4 \pm 3.1	214.3 \pm 23.0

^aData are expressed as means \pm SEM (n = 4). *p < 0.05, **p < 0.01 versus intact controls.

Table II. Inhibitory effects of 17 β -[N-alkyl/aryl]alkyl/arylamido]-4-methyl-4-aza-5 α -androstane-3-one series on the size of flank organ, ventral prostatic, and seminal vesicle weights. Compounds were applied on the right flank organ^a

EM (100 μ g, BID)	Flank organ surface area (mm ²)		Ventral prostate weight (mg)	Seminal vesicle weight (mg)
	Left side	Right side		
Control	33.2 \pm 2.0	33.6 \pm 1.6	55.4 \pm 3.1	236.3 \pm 13.2
424	31.9 \pm 1.3	32.8 \pm 1.6	56.5 \pm 4.4	265.4 \pm 15.8
486	26.3 \pm 0.6*	25.0 \pm 1.3**	59.3 \pm 7.4	267.1 \pm 16.6
497	30.4 \pm 1.2	30.4 \pm 0.8	59.1 \pm 2.8	249.3 \pm 5.3
494	31.1 \pm 1.1	30.7 \pm 0.9	53.8 \pm 3.3	240.1 \pm 5.3
493	31.7 \pm 2.1	27.3 \pm 1.4	57.8 \pm 2.0	238.3 \pm 6.3
498	30.4 \pm 1.6	32.3 \pm 2.1	53.1 \pm 3.5	248.8 \pm 8.3
503	31.9 \pm 1.2	30.0 \pm 1.0	55.1 \pm 1.6	277.1 \pm 11.2
580	30.2 \pm 1.1	32.1 \pm 1.2	53.7 \pm 3.2	224.2 \pm 4.8
568	23.2 \pm 0.7**	25.3 \pm 1.5**	45.7 \pm 3.5*	182.7 \pm 7.5**
606	23.5 \pm 2.3**	23.8 \pm 2.2**	40.3 \pm 3.4**	157.7 \pm 11.6**
567	24.0 \pm 1.6**	24.0 \pm 1.4**	51.3 \pm 1.4	211.8 \pm 6.9
682	28.1 \pm 1.8	31.1 \pm 1.4	42.9 \pm 2.0**	183.3 \pm 8.2**

^aData are expressed as means \pm SEM (n = 4). *p < 0.05, **p < 0.01 versus intact controls.

ventral prostates and seminal vesicles were not significantly reduced by treatment with any of the compounds used.

17 β -[(N-alkyl/aryl)alkyl/arylamido]-4-methyl-4-aza-5 α -androstane-3-ones systemic effect affect topical application A series of 17 β -[(N-alkyl/aryl)alkyl/arylamido]-4-methyl-4-aza-5 α -androstane-3-ones (Fig 1) were also tested on hamster flank organs. As illustrated in Table II, the topical administration of EM-486, EM-568, EM-606, and EM-567, at a dose of 100 μ g, twice daily, for 4 wk, caused respective 21% (p < 0.05), 30% (p < 0.01), 29% (p < 0.01), and 28% (p < 0.05) reductions in the left flank organ size and 26%, 25%, 29%, and 29% (p < 0.01 for all) reductions in the right side flank organ surface area compared with the intact control group. Ventral prostate weights were decreased by 18% (p < 0.05) and 27% (p < 0.01) following treatment with EM-568 and EM-606, respectively, whereas EM-486 did not affect this parameter. Moreover, treatment with EM-568 and EM-606, at the same dose, caused 23% and 33% (p < 0.01 for both) reductions in seminal vesicle weight, although EM-468 and EM-567 had no significant effect on this parameter. On the other hand, although EM-682 did not significantly affect flank organ surface area, ventral prostate and seminal vesicle weights were reduced by 23% and 22% (p < 0.01), respectively, with topical treatment at the twice daily dose of 100 μ g. As can be seen in Table II, treatment with EM-424, EM-497, EM-494, EM-498, EM-503, EM-493, and EM-580 did not affect the surface area of either flank organs or prostate and seminal vesicle weight.

EM-402 exerts potent local effects after topical application In order to obtain more precise information on the activity of the compounds potentially suitable for topical use and devoid of systemic

activity, we measured the effects of EM-401, EM-402, and EM-540 on the size of the flank organs and the size of the sebaceous glands of flank organs and ears, 5 α -reductase activity of flank organs and ears, as well as the weight of the prostate and seminal vesicle. The compounds were applied topically on the right flank organ and ear at doses of 30, 100, or 300 μ g, twice daily. As illustrated in Fig 2, the surface areas of the flank organs of intact control animals measured 32.7 \pm 1.0 and 33.4 \pm 1.22 mm² for the left and right sides, respectively. In hamsters treated with EM-401 at doses of 30, 100, and 300 μ g, twice daily, for 4 wk, left and right flank organs measured 28.1 \pm 1.1 and 29.2 \pm 1.7 mm², 26.0 \pm 1.4 and 25.0 \pm 1.5 mm², and 25.3 \pm 2.0 and 21.9 \pm 0.7 mm². On the other hand, treatment with increasing doses of EM-402 decreased the size of the right flank organ to the values of 26.0 \pm 1.2, 22.8 \pm 1.3, and 22.6 \pm 1.9 mm², whereas the size of left flank organs remained within the range of intact controls. Similar results were observed in the animals treated with EM-540 where right flank organ size decreased to 26.7 \pm 1.2, 25.3 \pm 1.6, and 20.7 \pm 1.0 mm² following doses of 30, 100, and 300 μ g, respectively, whereas the left flank organ did not change significantly.

Figure 3 illustrates the changes in the size of the sebaceous glands underlying the hamster flank organs that may be considered as a more clinically relevant parameter than the change in size of the flank organ itself. Treatment with 30, 100, and 300 μ g EM-401 reduced the size of the left flank sebaceous glands to 89.3% (not significant), 66.7% (p < 0.01), and 63.4% (p < 0.01) of control and that of the right flank sebaceous glands to 94.4% (not significant), 42.0% (p < 0.01), and 24.0% (p < 0.01) of control, respectively. In contrast, treatment with EM-402 and EM-540 reduced the size of the flank organ sebaceous glands on the treated side only, whereas contralateral glands

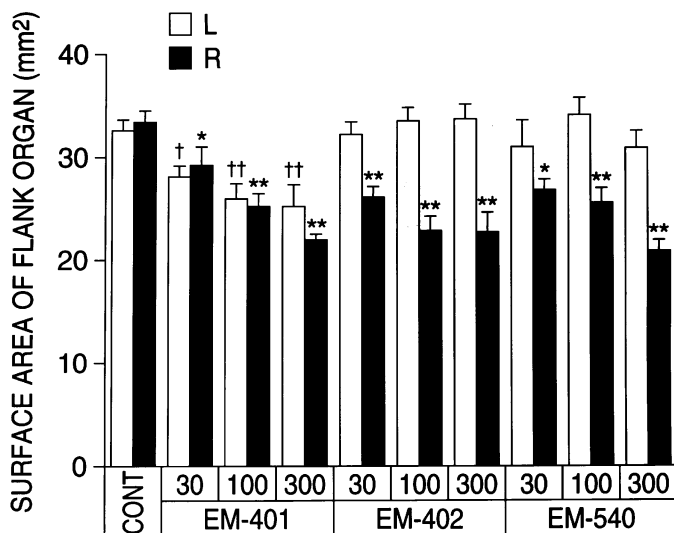


Figure 2. Treatment with EM-401, EM-402, or EM-540 specifically inhibits flank organ size after unilateral topical application. The right flank organ and ear were treated with doses of 30, 100, and 300 µg for 4 wk and the control and the left side of treated groups received vehicle only. †*p* < 0.05, ††*p* < 0.01 versus left side of control animals; **p* < 0.05, ***p* < 0.01 versus right side of control animals.

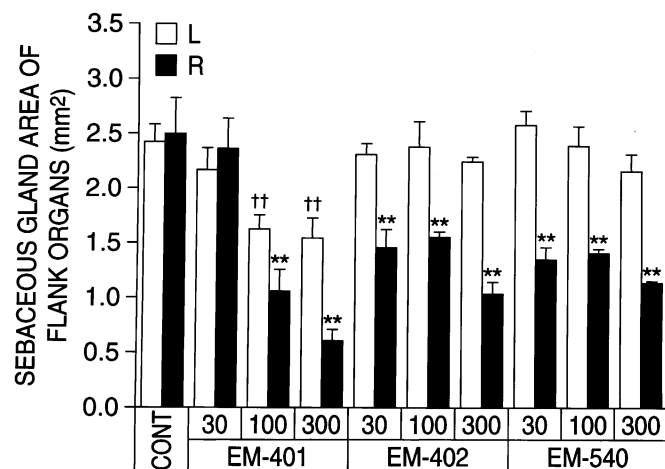


Figure 3. Treatment with EM-401, EM-402, or EM-540 specifically inhibits the size of sebaceous glands underlying flank organs after topical application. L, Left organ; R, right organ. ††*p* < 0.01 versus left side of control; ***p* < 0.01 versus right side of control.

were unaffected. In fact, EM-402 at the dose of 30 µg decreased right sebaceous gland size to 58% (*p* < 0.01) of control, whereas the doses of 100 and 300 µg twice daily, led to values of 61.6% and 40.8% (*p* < 0.01) of control, respectively. On the other hand, the inhibitory effect of EM-540 decreased right sebaceous gland size to 53% (*p* < 0.01) for the smallest dose used (30 µg), whereas the larger doses of 100 and 300 µg resulted in further decreases to 56% and 45% (*p* < 0.01) of control, respectively.

The inhibitory effects of EM-401, EM-402, and EM-540 were also examined on the size of the sebaceous glands of ears, because the inner surface of the hamster ear contains large sebaceous glands that are highly sensitive to androgens. As can be seen in **Fig 4**, the effects of the three compounds were comparable with those obtained on the size of the sebaceous glands of the flank organs. In fact, topical treatment with EM-401 at the dose of 30 µg decreased the size of sebaceous glands of the right ear to 57.6 ± 6.9% (*p* < 0.01) of control, whereas the left side was unaffected. Following twice daily treatment with 100 and 300 µg of EM-401, 91.4 ± 4.3% and 73.1 ± 6.2% (*p* < 0.01) of control values were measured on the left side and

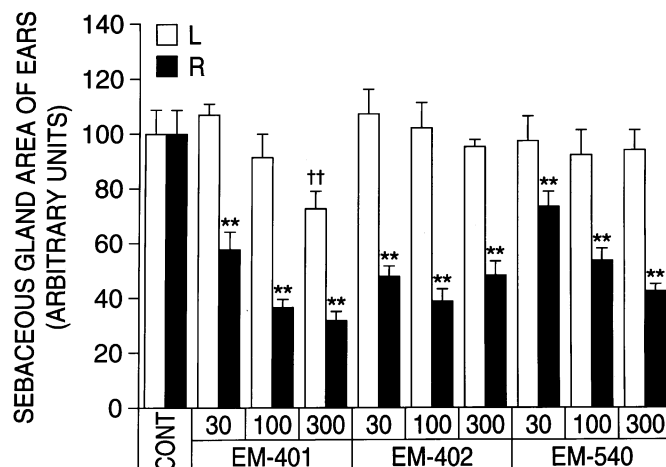


Figure 4. Treatment with EM-401, EM-402, or EM-540 specifically inhibits the size of ear sebaceous glands after topical application. The data are calculated in arbitrary units and expressed in percentages by setting the control group as 100%. L, Left; R, right. ††*p* < 0.01 versus left side of control; ***p* < 0.01 versus right side of control.

36.5 ± 3.1% and 31.9 ± 3.1% (*p* < 0.01) of control values were measured on the right side, respectively. On the other hand, EM-402 decreased right ear sebaceous gland size to 48.0 ± 3.6%, 38.9 ± 4.8%, and 48.8 ± 5.1% (*p* < 0.01) of control intact values and EM-540 decreased gland size to 73.8 ± 5.9%, 54.1 ± 4.7%, and 43.0 ± 2.2% at the doses of 30, 100 and 300 µg, respectively. Neither of the two compounds affected gland size in untreated ears.

As we reported previously, 17β-(N-alkyl/arylformamido)- and 17β-[(N-alkyl/aryl)alkyl/arylamido]-4-methyl-4-aza-5α-androstan-3-ones are potent inhibitors of transfected human type I 5α-reductase in DU-145 cells (Li *et al*, 1995). We therefore measured the inhibitory effect of EM-401, EM-402, and EM-540 on 5α-reductase activity of hamster flank organs as well as ears. As illustrated in **Fig 5(A)**, the left and right flank organs of intact animals display 5α-reductase activities of 1.45 ± 0.13 and 1.47 ± 0.09 pM DHT formed per mg protein per min. Treatment with EM-401 at the doses of 30, 100, and 300 µg reduced right flank organ 5α-reductase activity to 0.63 ± 0.07, 0.38 ± 0.02, and 0.22 ± 0.003 pM, respectively, and a significant reduction of left flank organ 5α-reductase activity to 0.82 ± 0.10 was observed at the dose of 300 µg only. Treatment with EM-402 decreased 5α-reductase activity to 0.78 ± 0.06, 0.30 ± 0.02, and 0.33 ± 0.09 pM in right flank organs, although left organ activity remained unchanged. On the other hand, EM-540 reduced contralateral flank organ 5α-reductase activity to 1.06 ± 0.11 pM and 0.94 ± 0.09 pM at the doses of 100 and 300 µg, respectively. Ipsilateral flank organ 5α-reductase activity was decreased to 1.07 ± 0.06, 0.51 ± 0.02, and 0.52 ± 0.09 pM at the doses of 30, 100, and 300 µg, respectively. Similar effects were observed on ear 5α-reductase activity except that all compounds exerted an inhibitory effect on the treated ears only (**Fig 5B**).

Ventral prostate and seminal vesicle weights were unaffected by 4 wk topical treatment with either EM-401, EM-402, or EM-540 at the doses used (data not shown).

DISCUSSION

These data show that 17β-(N-alkyl/arylformamido)- and 17β-[(N-alkyl/aryl)alkyl/arylamido]-4-methyl-4-aza-5α-androstan-3-ones are potent antagonists of androgen action in hamster flank organs and ears. Among the series of compounds tested, EM-402 [17β-(N-hexylformamido)-4-methyl-4-aza-5α-androstan-3-one] is the most potent inhibitor for local use. In fact, the topical administration of EM-402 reduced the size of flank organs and of underlying sebaceous glands and the size of ear sebaceous glands, and inhibited 5α-reductase activity in flank organ and ear tissues. Contralateral organs as well as the prostate and seminal vesicle were unaffected following treatment with doses of 30, 100, and 300 µg, twice daily for 4 wk.

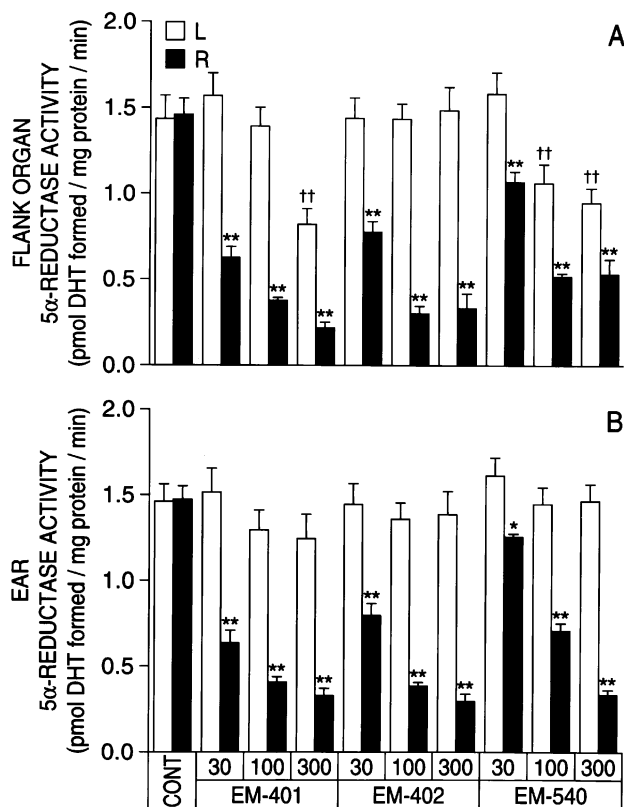


Figure 5. Treatment with EM-401, EM-402, or EM-540 inhibits 5 α -reductase activity in flank organ (A) and ear (B) after topical application. ⁺⁺p < 0.01 versus left side of control; ^{**}p < 0.01 versus right side of control.

EM-402 inhibits human type I 5 α -reductase activity at an IC₅₀ value of 7.25 \pm 0.82 nM, a value higher than that of EM-401 [17 β -(N-amyloformamido)-4-methyl-4-aza-5 α -androstane-3-one] measured at 0.91 \pm 0.24 nM in transfected cells *in vitro* (Li *et al.*, 1995). This study also shows that EM-401 is a somewhat more potent inhibitor than EM-402 for the 5 α -reductase present in the flank organs and ears of the hamster (Fig 5). The inhibitory effect of EM-401 was observed on both the treated and untreated sides after topical administration in a dose-dependent manner, thus suggesting that EM-401 exerts some systemic effects after topical application on the flank organ in the hamster. The inhibitory effect of EM-401 was observed at the 100 μ g dose on the size of the sebaceous glands of the flank organ whereas the effect of the compound on 5 α -reductase activity was seen only at the 300 μ g dose: this apparent discrepancy could possibly be explained by the dilution of the compound in the 5 α -reductase assay. No obvious explanation is available, on the other hand, to take into account the inhibition of 5 α -reductase activity in the left flank organ in EM-540-treated animals and the effect on sebaceous gland size is not significant. EM-540 [17 β -(N-heptylformamido)-4-methyl-4-aza-5 α -androstane-3-one], on the other hand, reduced the size of the flank organs and associated sebaceous glands, as well as that of the ear sebaceous glands on the treated side only; however, a small inhibitory effect on 5 α -reductase activity was observed in the untreated flank organ but not in the ears. Because the structures of the 5 α -reductase isoenzymes in the hamster are not known, it was important to compare the inhibitory activity of the compounds on hamster sebaceous gland 5 α -reductase as well as on the isoenzyme expressed in the human sebaceous glands, namely type 1 5 α -reductase (Li *et al.*, 1995).

For the 17 β -(N-alkyl/arylformamido)- series, those compounds having a number (n) of carbon atoms in a N-alkyl chain between four and eight, such as linear N-alkyl EM-347 (n = 4), EM-401 (n = 5), EM-402 (n = 6), EM-540 (n = 7), EM-541 (n = 8), and the branched N-alkyl EM-435 (n = 6), as well as the N-aryl EM-436, significantly reduced the size of hamster flank organs. In addition, most of the

above-mentioned compounds exclusively have topical effects, except EM-401 and EM-541 that show some systemic effects. When the N-substitution is a cyclohexyl, like in EM-337, little effect is observed. Furthermore, if the N-substitution possesses a cyclopropyl group, the compound ends up with weak or no effect, such as in EM-336 (n = 3). On the other hand, the replacement of the proton (H) of N-formaldehyde (RNCOH) of 17 β -(N-alkyl/arylformamido)- by an alkyl or aryl (R¹) giving 17 β -[(N-alkyl/aryl)alkyl/arylamido]- series (RNCOR¹), of which one (N-alkyl)alkylamido-compound (EM-486) and three (N-aryl)alkylamido-compounds (EM-567, EM-568, and EM-606) also significantly reduce the size of flank organ but all show systemic effects. In comparison with the parent compound (EM-568), an electron donating group (EM-606, 4'-OCH₃) or an electron withdrawing group (EM-567, 4'-NO₂) substituted on the aromatic ring does not translate into effects on the inhibitory potency and topical/systemic selectivity. The introduction of a bromine atom at the end of an alkyl formamido group (EM-486 *vs* EM-493) significantly reduces the size of both side flank organs. The remaining compounds of this series, such as EM-424, EM-493, EM-494, EM-497, EM-498, EM-503, and EM-580, however, have very weak or no effect on the measured parameters. Consequently, the structure-activity relationships of these compounds remain to be determined.

All hamsters, male and female, have an asymmetrical pair of pigmented flank organs (also called costovertebral glands) on their backs that are made up of three androgen-dependent structures: pigment cells, hair follicles, and sebaceous glands (Hamilton and Montagna, 1950). Systemic and local effects of topical anti-androgens can be appropriately studied using this model (Matias and Gaillard, 1995). The presence of paired organs provides a built-in control for the study of topical applications made on one side. Although it had been assumed that the three target tissues – pigmentation, hair, and sebaceous glands – were equally responsive to androgens, recent studies have shown that each may indeed be under separate androgen control (Vermorken *et al.*, 1980, 1982). Thus, a compound that inhibits the sebaceous glands may not have the same effect on hair. For instance, earlier studies of the pigmented spot overlying the flank organ have been used as a measure of the androgen response, but we now know that this color change does not necessarily reflect sebaceous gland or hair follicle response. One possible explanation could be the presence of different 5 α -reductase isoenzymes in these different structures. These data show that EM-402 decreased the size of the pigmented area by 22% to 32% and the size of the underlying sebaceous glands by 42% to 59% in the dorsal flank organs after treatment with 30, 100, and 300 μ g, twice daily. These findings suggest that the observation of an inhibition of the sebaceous gland development by inhibitors is a more specific and precise parameter than changes of the pigmentation spot. It is also interesting to mention that a dose-dependent decrease of ear sebaceous gland area was obtained on ear sebaceous gland area following treatment with EM-401 and EM-540, whereas a maximal inhibitory effect was observed at the lowest dose of EM-402 used.

As 5 α -reductase inhibitors do not block the binding of testosterone to its receptor, these agents have not been reported to cause reproductive problems in human males (Rittmaster, 1988; Stoner, 1990). Previously, Rittmaster *et al.* have reported that a 5 α -reductase inhibitor, N,N-diethyl-4-methyl-3-oxo-4-aza-5 α -androstane-17 β -carboxamide (4-MA), prevented baldness when administered topically to the stump-tail macaque (Rittmaster *et al.*, 1987). Oral treatment with N-(1,1-dimethyl-ethyl)-3-oxo-4-aza-5 α -androstane-1-ene-17 β -carboxamide (MK-906, Finasteride), another 5 α -reductase inhibitor, reversed the balding process and enhanced hair re-growth in combination with topical minoxidil in the male balding stump-tail macaque (Diani *et al.*, 1992). Both of these compounds, however, exert systemic effects after topical application as indicated by the inhibition of prostate growth (Labrie *et al.*, 1991; Imperato-McIngle *et al.*, 1992; Martel *et al.*, 1994; Chen *et al.*, 1995). In this respect, locally acting 5 α -reductase inhibitors, such as EM-402, could be advantageous for the treatment of localized androgen-sensitive diseases. It should also be mentioned that despite the high efficiency of 5 α -reductase inhibitors as inhibitors of DHT formation, it seems logical that these compounds should not be administered alone in the treatment of androgen-sensitive cancer, due

to the secondary increase in intratissular testosterone concentration that is likely to partially overcome the blockade achieved by the 5 α -reductase inhibitor (Labrie *et al*, 1991; Martel *et al*, 1993; Chen *et al*, 1995). In fact, testosterone itself binds to the androgen receptor with a K_d value close to that of DHT and is capable of fully activating the androgen receptor (Grino *et al*, 1990). The inhibition of androgen action could be best achieved by combining a 5 α -reductase inhibitor and an anti-androgen.

An ideal topical compound should exert local anti-androgenic effects exclusively. Although the oral administration of systematically active pure anti-androgens such as flutamide is an effective treatment (Cusan *et al*, 1990, 1993, 1994; Labrie *et al*, 1996), it is not an ideal choice in practice because of the possibility that such drugs might impair spermatogenesis in men and feminize male fetuses in pregnant women. These data clearly demonstrate that EM-402 is a potent 5 α -reductase inhibitor exerting local anti-androgenic effect. These findings should encourage a more detailed study of the mechanisms underlying EM-402 metabolism and action. In addition, compounds such as EM-402 could be considered as suitable candidates for assessment of their activity in human clinical trials.

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