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SHORT REPORT

Identification of steroid derivatives that function as potent antiandrogens

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We have hypothesized that some steroid derivatives bind to the androgen receptor (AR) with very low androgenic activity and therefore potentially function as better AR antagonists than clinically used antiandrogens, such as flutamide. Indeed, we previously found such a compound, 3β-acetoxyandrosta-1,5-diene-17-one ethylene ketal (ADEK), with some estrogenic activity. Here we report the identification of 2 additional steroid derivatives, 3β-hydroxyandrosta-5,16-diene (HAD) and androsta-1,4-diene-3,17-dione-17-ethylene ketal (OAK), as new potent antiandrogens. Like ADEK, HAD and OAK could interrupt androgen binding to the AR and suppress both dihydrotestosterone- and androstenediol-induced transactivations of wild-type and mutant ARs in prostate cancer cells. These 2 compounds also inhibited prostate-specific antigen expression in LNCaP as well as growth of different AR-positive prostate cancer cell lines stimulated by androgen. Significantly, HAD and OAK had only marginal agonist effects, as compared to hydroxyflutamide. More importantly, in contrast to ADEK, OAK was shown to possess marginal estrogenic activity. These results strengthen our hypothesis and suggest that selective steroid derivatives could be potent antiandrogenic drugs with less unfavorable effects for the treatment of prostate cancer.

Key words: androgen receptor, antiandrogens; prostate cancer; antiandrogen withdrawal syndrome

Since the growth of prostate cancer is generally dependent on androgen stimulation, androgen deprivation therapy represents the most effective treatment for patients with advanced prostate cancer. Although multiple approaches have been used to reduce the bioavailability of androgens or interfere with their function *via* the androgen receptor (AR), combined androgen blockade (CAB), consisting of surgical castration or the administration of a luteinizing hormone-releasing hormone analogue combined with an AR antagonist, is a standard option (reviewed in reference 1). Nonetheless, no curative treatment exists for patients with metastatic prostate cancer. After initial regression, almost all responders to the hormonal therapy ultimately appear to lose the dependency that results in tumor progression. In addition, before this transition from an androgen-dependent to androgen-independent phenotype, AR antagonists sometimes promote disease progression, mostly as an increase in serum levels of prostate-specific antigen (PSA), the most useful tumor marker and also an AR-responsive gene. In these patients, discontinuation of AR antagonists results in a significant fall in PSA values, often correlated with clinical improvement. This phenomenon, known as antiandrogen withdrawal syndrome or steroid hormone withdrawal syndrome, has been observed with the majority of clinically used antiandrogens, including flutamide and bicalutamide (BC), as well as some hormone mimics, such as the synthetic estrogen diethylstilbestrol (DES) and a progestational agent magestrol.[2,3] The molecular basis for this syndrome is not completely understood, but potential mechanisms (reviewed in reference 3) include 1) AR gene mutations, such as a point mutation at codon 877[4] or at 741[5]; 2) AR coregulatory protein alterations, such as overexpression of gelsolin[6] or ARA70[7] and 3) activation of mitogen-activated protein kinase pathway by antiandrogens.[8] Clearly, antiandrogens (AR antagonists) act as agonists in these cases.

Dehydroepiandrosterone (DHEA) is produced abundantly in human adrenal glands and is converted metabolically into androstenediol (Adiol), androstenedione (Adione), testosterone (T), dihydrotestosterone (DHT) and a number of different steroids.[9,10] We have found that ''adrenal androgens'', precursors of T, including Adiol and Adione, possess intrinsic androgenic activity that was not blocked by hydroxyflutamide (HF) and BC in prostate cancer cells.[9,11] Because castration, with or without antiandrogen, reduces the serum concentration of adrenal androgens by only 40–50%,[12,13] our findings[9,11] suggested a reason for the failure of therapy to prevent AR-positive prostate cancer growth during the androgen-independent state.

Our proposal is supported by the recent report[14] that Adiol is concentrated in the prostate gland and that it is a more effective activator of mutant AR than DHT. We have synthesized a number of steroids and tested their antiandrogenic activity *vs*. Adiol and DHT.[15,16] The most effective was 3β-acetoxyandrosta-1,5-diene-17-one ethylene ketal (ADEK), which inhibits both DHT- and Adiol-induced AR transcription, PSA expression and growth in prostate cancer cells. Significantly, ADEK has marginal androgenic activity and a strong AR coactivator, ARA70, which has been shown to enhance agonist activity of clinically used antiandrogens,[17,18] fails to induce it. However, ADEK was shown to possess estrogenic activity, which will require monitoring to determine whether it will cause side effects such as cardiovascular events as in the case of DES.

Our study was undertaken to seek better antiandrogens with marginal androgenic and estrogenic activities. We screened a number of synthesized steroid derivatives and found 2 antiandrogenic compounds with suitable properties.

Material and methods

Chemicals and plasmids

pSG5-AR, pSG5-ARA70, pSG5-gelsolin and mouse mammary tumor virus (MMTV)-luciferase (Luc) reporter were used in our previous studies.[6,9,11,16,17] PSA-Luc containing a natural 6.0 kb promoter and a synthetic (ARE)4-Luc containing 4 copies of androgen response element (ARE) were kindly provided by Dr. A. Mizokami (Kanazawa University, Kanazawa, Japan) and Dr. M.L. Lu (Harvard Medical School, Boston, MA), respectively. DHT, Adiol, progesterone (Prog), dexamethasone (Dex) and 17β-estra-

Abbreviations: ADEK, 3β-acetoxyandrosta-1,5-diene-17-one ethylene ketal; Adiol, androstenediol; Adione, androstenedione; AR, androgen receptor; ARE, androgen response element; BC, bicalutamide; CAB, combined androgen blockade; DES, diethlystilbestrol; Dex, dexamethasone, E2, 17β-estradiol; ETOH, ethanol; FBS, fetal bovine serum; GR, glucocorticoid receptor; HAD, 3β-hydroxyandrosta-5,16-diene; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; ER, estrogen receptor; HF, hydroxyflutamide; Luc, luciferase; MMTV, mouse mammary tumor virus; OAK, 3-oxo-androsta-1,4-diene-17-ethylene ketal; Prog, progesterone; PR, progesterone receptor; PSA, prostate-specific antigen; RBA, relative binding affinity; T, testosterone; WT, wild-type.

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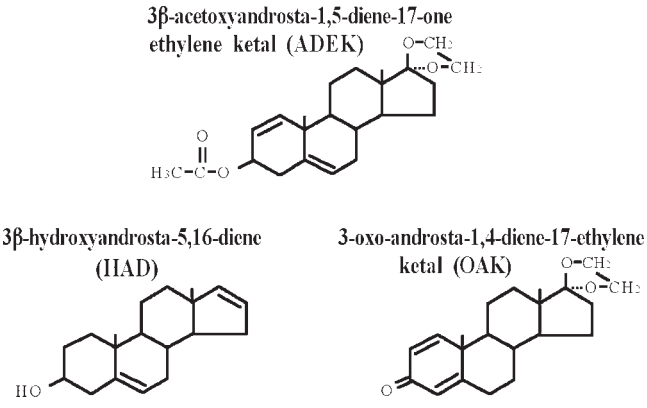

FIGURE 1 – The structures of steroid derivatives.

diol (E2) were obtained from Sigma Chemical Co. (St. Louis, MO). HF was from Schering, and BC was from ICI. Other steroid compounds, derivatives of DHEA, were synthesized; some have been described.[19] The steroids tested include 3β-hydroxyandrosta-5,16-diene (HAD) and androsta-1,4-diene-3,17-dione-17-ethylene ketal (OAK), the activities of which are described here and the following which were not effective: 3β,17β-diacetoxy-17α-ethynyl-androsta-1,5-diene; 17α-ethynyl-androsta-1,5-diene-3β, 17β-diol; androst-5-ene-3,17-dione-diethylene ketal; 3β,16α-dicarbomethoxy androst-5-en-17-one; 3β,16β-diacetoxyandrost-5-en-17-one; 3β,17β-dihydroxyandrost-5-en-16-one; 3β,7β-dihydroxy-16,16-seco-androst-5-ene-16,17-dioic acid(16 → 7) lactone; 3β-acetoxy-17α-oxa-D-homo-androst-5-en-17-one; 3β,13α-dihydroxyandrost-5-ene-D-seco-14β-propionic acid. All the compounds were dissolved in ethanol (ETOH) and dilutions of 1 μm–1 mM were prepared.

Cell culture, transfection and reporter gene assay

The human prostate cancer cell lines, LNCaP, PC-3, and CWR22R, and nonprostate cancer cell line COS-1 were maintained in RPMI or Dulbecco's modified Eagle's medium (Life Technologies, Bethesda, MD) supplemented with 10% fetal bovine serum (FBS). Transfections and Luc assays were performed as previously described.[17,20] Briefly, cells seeded to reach a density of 50–60% confluence in 12-well tissue culture plates were transfected with 1.5 μg of DNA according to ''SuperFect transfection'' instructions (Qiagen, Chatsworth, CA). After 2–3 hr incubation, cells were cultured in medium supplemented with charcoal-stripped FBS and either 0.1–0.2% (vol/vol) ETOH or ligands for 24 hr. The cells were then harvested, and whole cell extracts were used for Luc assay. The Luc activity was determined using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI) and luminometer.

MTT (thiazolyl blue) assay

MTT assay was used to assess cell growth, as described previously.[21] Cells that were seeded in 6-well tissue culture plates were incubated with medium supplemented with charcoal-stripped FBS containing either ETOH or ligands. After 6 days of treatment (changing media every 2 days), 200 μl of MTT (Sigma Chemical Co.) stock solution (5 mg/ml) was added into each well with 2 ml of medium for 3 hr at 37°C. Then, 1–2 ml of 0.04 N HCl in isopropanol was added for 5 min incubation at room temperature. Absorbency was read at a wavelength of 570 nm with background wavelength at 660 nm.

Western blot

Western blotting analysis was performed in LNCaP cells, using monoclonal PSA antibody (DAKO), as described previously.[20] An antibody for β-actin (Santa Cruz Biotechnology, Santa Cruz, CA) was used as the internal control. Equal amounts of proteins obtained from cell extracts were electrophoresed on SDS-polyacrylamide gel and blotted onto polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA), using a standard protocol. The detection of the specific antibodies was accom-

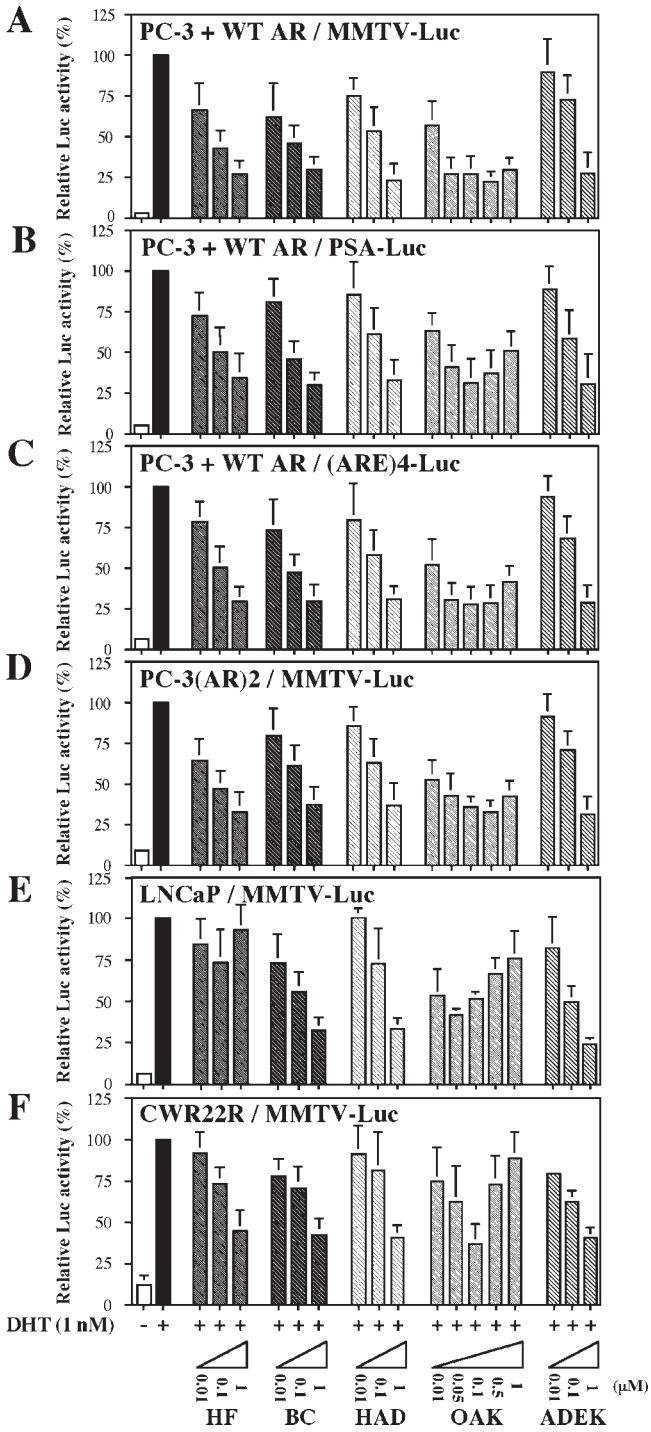

FIGURE 2 – The effects of steroid derivatives on the DHT-induced transcriptional activity of AR. PC-3 (*a–c*), PC-3(AR)2 (*d*), LNCaP (*e*) or CWR22R (*f*) cells were transfected with MMTV-Luc (*a*, *d–f*), PSA-Luc (*b*) or (ARE)4-Luc (*c*). The WT AR expression plasmid pSG5-AR was cotransfected in PC-3 cells (*a–c*). After transfection, cells were cultured for 24 hr in the presence of 1 nM DHT and various concentrations of 1 μM HF, 1 μM BC, HAD, OAK or ADEK as indicated. The Luc activity is presented relative to that in the presence of DHT in each panel (black bars; set as 100%). Values represent the mean ± SD of at least 3 determinations.

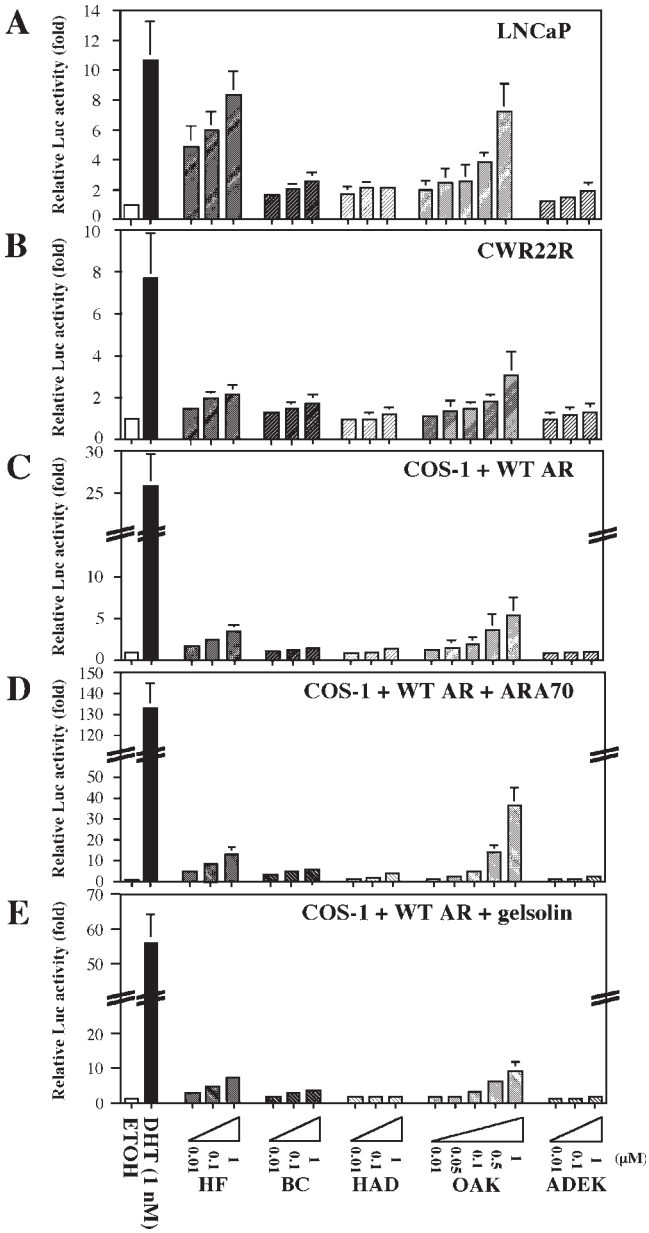

FIGURE 3 – The effects of steroid derivatives on the transcriptional activity of AR. LNCaP (*a*) or CWR22R (*b*) cells were transfected with MMTV-Luc. COS-1 cells were transfected with the pSG5-AR and MMTV-Luc in the presence of pSG5 vector (*c*), pSG5-ARA70 (*d*) or pSG5-gelsolin (*e*). After transfection, cells were cultured for 24 hr with 1 nM DHT or various concentrations of 1 μM HF, 1 μM BC, HAD, OAK or ADEK, as indicated. The Luc activity is presented relative to that of ETOH treatment (without ARA70) (white bars; set as 1-fold). Values represent the mean ± SD of at least 3 determinations.

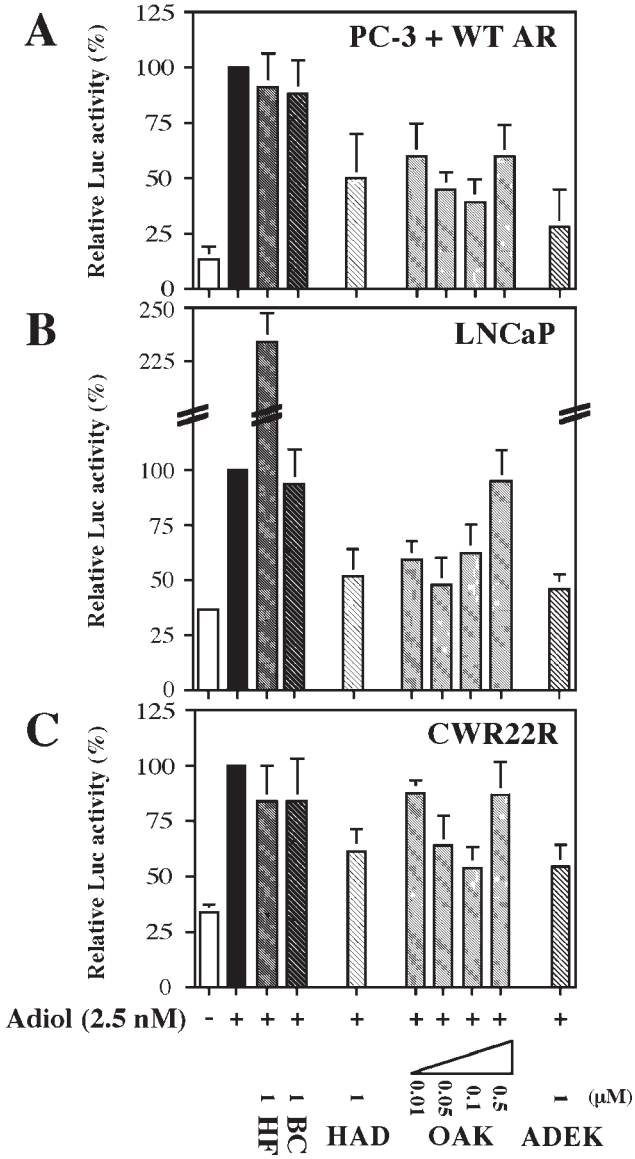

FIGURE 4 – The effects of steroid derivatives on the Adiol-induced transcriptional activity of AR. PC-3 (*a*), LNCaP (*b*) or CWR22R (*c*) cells were transfected with MMTV-Luc. The pSG5-AR was cotransfected in PC-3 cells. After transfection, cells were cultured for 24 hr in the presence of 2.5 nM Adiol and 1 μM HF, 1 μM BC, 1 μM HAD, 0.01–0.5 μM OAK or 1 μM ADEK, as indicated. The Luc activity is presented relative to that in the presence of Adiol in each panel (black bars; set as 100%). Values represent the mean ± SD of at least 3 determinations.

plished using alkaline phosphatase detection systems (Bio-Rad, Richmond, CA).

Ligand binding assay

Whole cell extracts from COS-1 with transient transfection of pSG5-AR, or LNCaP without transfection, were incubated for 2 hr at 37°C with 1 nM [^{3}H]-synthetic androgen methyltrienolone (R1881) in the presence and absence of increasing concentrations (1–10,000 nM) of unlabeled ligands. Hydroxyapatite (Bio-Rad) was then added and stirred for 15 min at 4°C. After centrifugation and washing, radioactivity was determined by scintillation counting.

Statistical analysis

The differences in cell growth were analyzed by the Student's *t*-test; *p* values less than 0.05 were considered to be statistically significant.

Results and discussion

Anti-DHT effect of steroid derivatives on AR transcription

Using a reporter gene assay, we first investigated the ability of newly synthesized steroid derivatives to inhibit DHT-induced AR transcriptional activity in the AR-negative PC-3 cell line. The Luc activity was determined in the cell extracts with transient

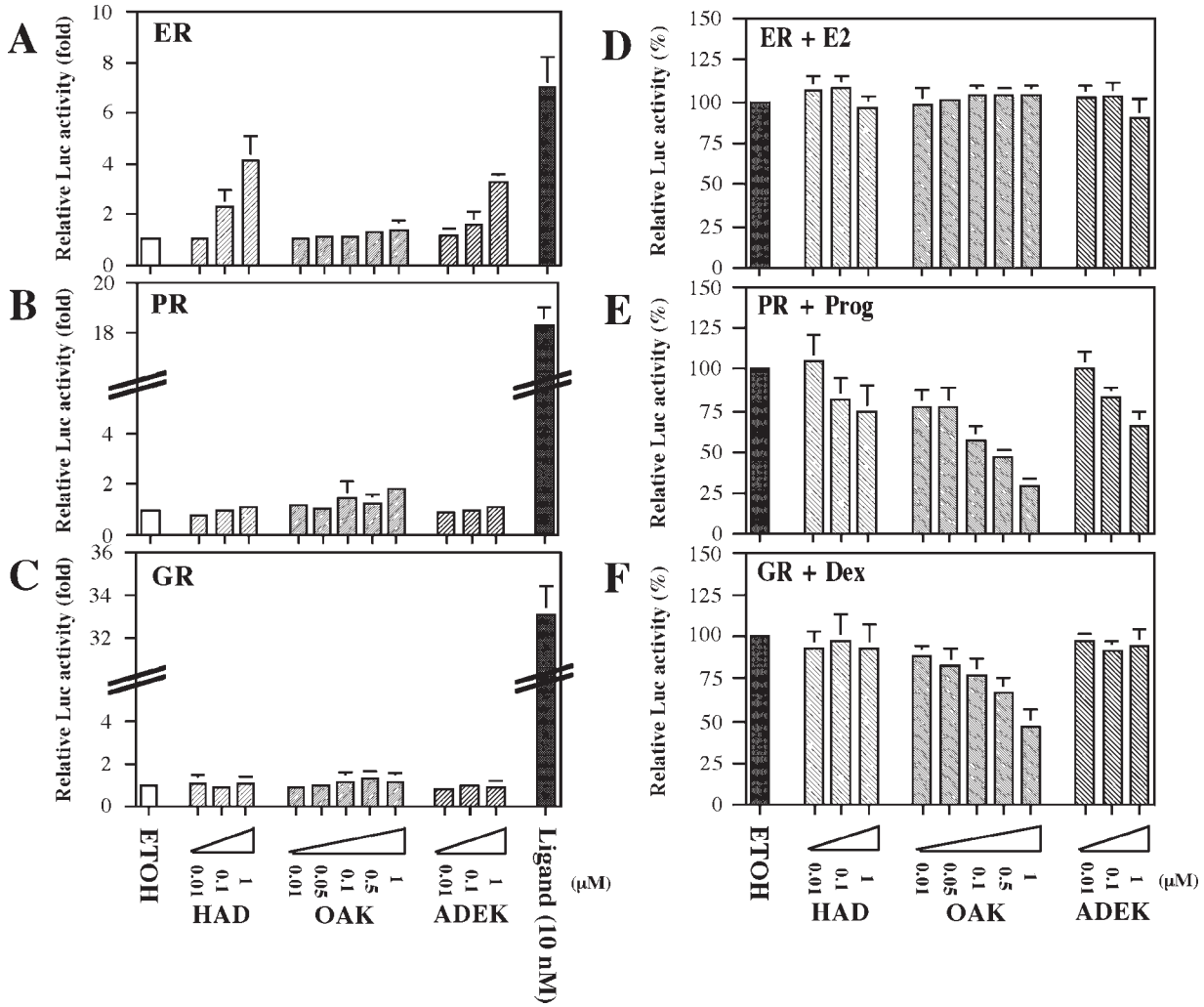

FIGURE 5 – The effects of steroid derivatives on the transcriptional activity of ER (*a*,*d*), PR (*b*,*e*), and GR (*c*,*f*). PC-3 cells were transfected with steroid receptor and its reporter (PR/MMTV-Luc, GR/MMTV-Luc and ER/ERE-Luc). After transfection, cells were cultured for 24 hr in the absence (*a–c*) or presence (*d–f*) of ligand (10 nM Prog, 10 nM Dex or 10 nM E2) and increasing concentrations of ADEK, HAD or OAK, as indicated. The Luc activity is presented relative to that of ETOH treatment in each panel (white bars; set as 1-fold). Values represent the mean ± SD of at least 3 determinations.

transfection of wild-type (WT) AR plasmid and an ARE-reporter plasmid (MMTV-Luc). After transfection, the cells were treated with 1 nM DHT and each steroid derivative at 1–1,000 nM. Of 11 compounds tested, only 2 (HAD and OAK, see Fig. 1) exhibited anti-DHT activity on AR transcription (data not shown). These 2 compounds were further investigated and were also compared to ADEK, HF and BC. In PC-3 cells with MMTV-Luc, HAD at 1 μM and OAK at 0.05–1 μM suppressed DHT-induced WT AR transcription to approximately 20%, similar to the suppression by 1 μM HF, 1 μM BC or 1 μM ADEK (Fig. 2*a*). Similar suppressions were observed when MMTV-Luc was replaced with PSA-Luc (Fig. 2*b*) or (ARE)4-Luc (Fig. 2*c*) in PC-3 cells or when PC-3(AR)2 (PC-3 with stable transfection of WT AR under control of a cytomegalovirus promoter)[22] (Fig. 2*d*) or PC-3(AR)9 (PC-3 with stable transfection of WT AR under control of a natural AR promoter; Altuwaijri *et al*., unpublished data) (figure not shown) was used. None of these compounds as well as DHT, HF and BC displayed their effects on AR transcriptional activity in PC-3 without transfection of AR. In the LNCaP cell line expressing a mutant AR, HF is known to act as a full agonist[11,20,23] and therefore shows little suppression of DHT-induced AR transcription (Fig. 2*e*). However, 1 μM HAD and 0.05 μM OAK, as well as 1 μM BC and 1 μM ADEK, still exhibit suppression to <40%. In another AR (mutated)-positive prostate cancer cell line (CWR22R), HF, BC and 3 steroid derivatives show >50% suppression (Fig. 2*f*). These results suggest that HAD and OAK function as potent antagonists on DHT-enhanced transactivation of both WT AR and mutant ARs.

Agonist effect of steroid derivatives on AR transcription

Androgenic activity of HAD and OAK was then investigated. Cells transfected with MMTV-Luc (and WT AR only in COS-1) were incubated in the presence of each steroid derivative, and Luc activity was measured. As shown in Figure 3*a*, HAD and OAK (up to 0.1 μM) have low (<3-fold) androgenic activity on AR transcription in LNCaP cells, whereas high concentration (1 μM) of OAK shows induction similar to that by 1 μM HF. Similarly, HAD and OAK (up to 0.1 μM) have lower androgenic activity on AR transcription than HF (or BC) in CWR22R (Fig. 3*b*) and COS-1 transfected with WT AR (Fig. 3*c*). Biphasic effects of OAK on DHT-induced AR transcription observed in LNCaP (Fig. 2*e*) or CWR22R (Fig. 2*f*) may be attributable to the androgenic activity of OAK at higher concentrations (0.5–1 μM). In addition, 2 AR coactivators, ARA70 and gelsolin, significantly enhance DHT- and HF- or BC-mediated AR transactivation (3–5-fold) but marginally (<3-fold) enhance AR transactivation in the presence of

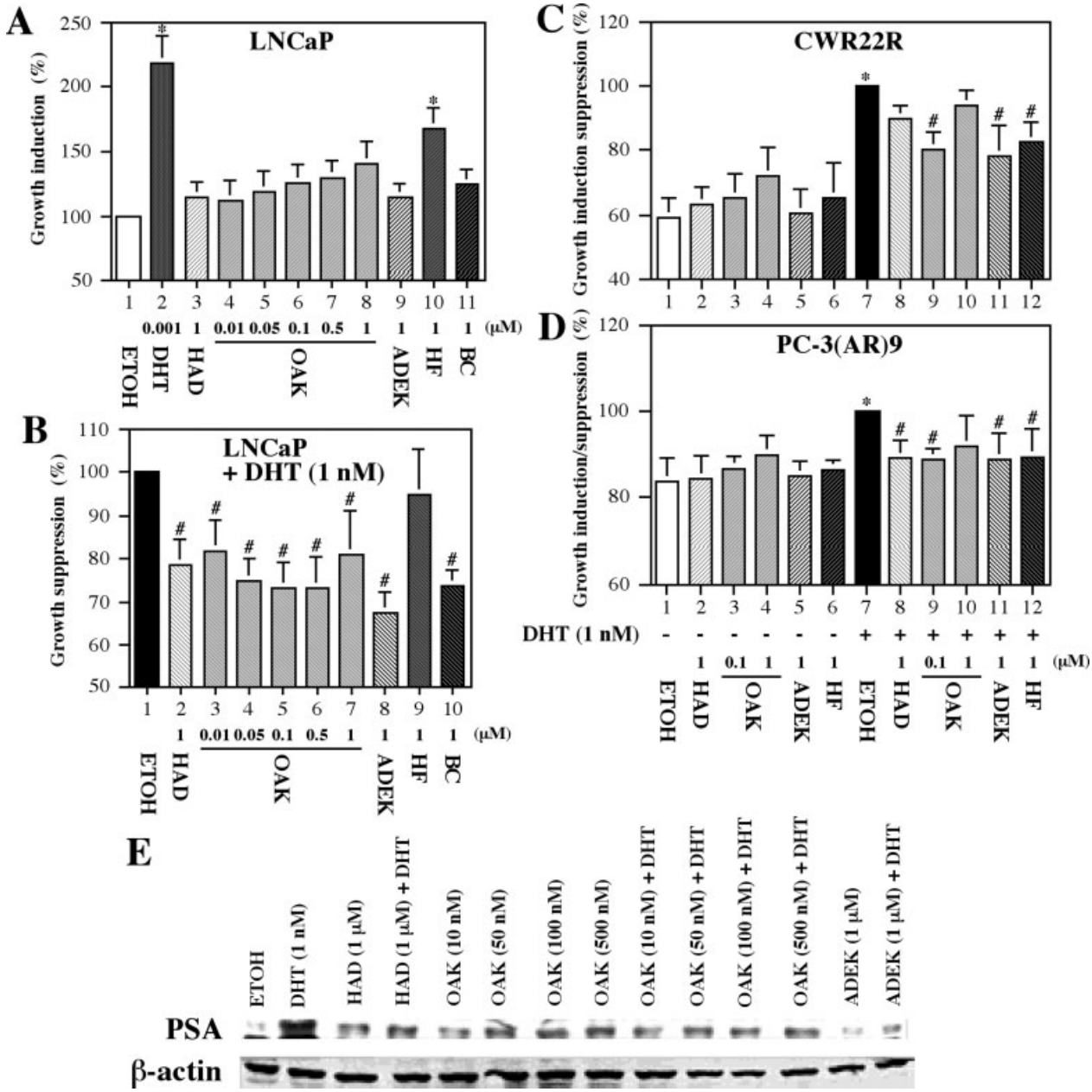

FIGURE 6 – The effects of steroid derivatives on cell proliferation and PSA expression. LNCaP (*a*,*b*), CWR22R(*c*), and PC-3(AR)9 (*d*) cells were cultured with 1 μM HF, 1 μM BC, 1 μM HAD, 0.01–1 μM OAK or 1 μM ADEK in the absence or presence of 1 nM DHT for 6 days, as indicated. The MTT assay was performed, and growth induction/suppression are presented relative to cell number with ETOH treatment (*a*; white bar; set as 100%) or DHT treatment alone (*b–d*; black bars; set as 100%). Values represent the mean ± SD of at least 3 determinations. *$p < 0.05$ (*vs*. ETOH), #$p < 0.05$ (*vs*. DHT). (*e*) Cell extracts from LNCaP cells cultured for 48 hr with 1 μM HAD, 10–500 nM OAK or 1 μM ADEK in the presence or absence of 1 nM DHT as indicated, were analyzed on Western blots using an antibody to the PSA. The 33 kDa protein was detected as indicated. β-Actin expression was used as an internal control.

ADEK, HAD and OAK (up to 0.1 μM) (Fig. 3*c vs*. Fig. 3*d* or 3*e*). Similar results were also obtained when MMTV-Luc was replaced with PSA-Luc (figure not shown). Thus, the agonist effect of HAD and OAK (up to 0.1 μM) is marginal and is not enhanced by AR coactivators that may play a key role in inducing antiandrogen withdrawal syndrome.[3,6,7,17] These findings suggest that HAD and OAK, as well as ADEK, carry fewer risks of withdrawal response in prostate cancer patients.

Anti-Adiol effect of steroid derivatives on AR transcription

We previously showed that Adiol, which is produced from DHEA and can be converted to T, also possesses intrinsic androgen activity, and that both HF and BC fail to significantly block Adiol-induced AR transactivation in prostate cancer cells.[9] We then found that ADEK has anti-Adiol effect on AR transcription in prostate cancer cells.[16] In our study, we also measured MMTV-Luc activity to determine whether HAD and OAK inhibit Adiol-induced AR transcription. Adiol at 2.5 nM increases AR transcriptional activity in PC-3 (Fig. 4*a*), LNCaP (Fig. 4*b*) and CWR22R (Fig. 4*c*) to 3–6-fold over mock treatment. HAD (1 μM) and OAK (0.05 or 0.1 μM) repress Adiol-induced AR transcription up to 49% and 39%, respectively, which is similar to the suppression by ADEK, whereas HF and BC fail to block it significantly. These results demonstrate that

HAD and OAK can suppress AR transactivation induced by both testicular and adrenal androgens.

Hormone specificity of steroid derivatives

We showed that many DHEA metabolites, including ADEK, possess some estrogen activity,[15,16] suggesting that ADEK might cause not only castration effect but also some side effects, such as cardiovascular toxicity *in vivo*. Because of these unfavorable side effects, estrogens (*e.g.*, DES) are rarely used as first-line hormonal treatment in spite of the evidence suggesting an additional benefit, a direct cytotoxic effect of estrogens through both estrogen receptor (ER)-dependent and ER-independent pathways in prostate cancer cells.[1] Hence, a purpose of further screening of steroid derivatives in our study is to identify antiandrogenic compounds that have effects similar to (or better than) ADEK, without any steroid hormone activity. To assess steroid hormone activity of HAD and OAK, PC-3 cells were transfected with steroid receptors/reporter (progesterone receptor (PR)/MMTV-Luc, glucocorticoid receptor (GR)/MMTV-Luc, or ER/ERE-Luc). As shown in Figure 5*a*, HAD has some estrogenic activity, which is stronger than that of ADEK. In contrast, OAK has only marginal estrogenic activity. These compounds have no Prog (Fig. 5*b*) or glucocorticoid (Fig. 5*c*) activity. Antagonist activity of these 3 steroids for each receptor was also examined. All the compounds exhibit marginal anti-ER activity (Fig. 5*d*), whereas each, especially OAK, significantly inhibits Prog-induced PR transcription (Fig. 5*e*). OAK also shows suppression on Dex-induced GR transcription to 77% at 0.1 μM and 46% at 1 μM (Fig. 5*f*).

Anti-DHT effect of steroid derivatives on cell growth and PSA expression

We next tested the effect of HAD and OAK on cell growth of AR-positive prostate cancer lines. As shown in Figure 6*a*, in LNCaP DHT (1 nM) significantly increases cell growth (lanes 1 *vs*. 2), and HAD (1 μM) and OAK (0.01–0.05 μM) show only slight (<20%) induction (lanes 3–8), similar to that by ADEK (lane 9), in the absence of androgens. Then, HAD and OAK significantly antagonize the DHT effect (Fig. 6*b*, lanes 1 *vs*. 2–8). In CWR22R cells whose growth has been reported to be stimulated by androgen,[24] DHT increases the growth by approximately 70% (Fig. 6*c*, lanes 1 *vs*. 7). HAD (1 μM; $p = 0.0560$) and OAK (0.1 μM; $p < 0.05$), as well as ADEK (1 μM; $p < 0.05$) and HF (1 μM; $p < 0.05$), showing no significant growth induction in the absence of androgens (lanes 1 *vs*. 2–6), suppress DHT-stimulated cell growth (lanes 7 *vs*. 8–12). Similarly, in PC-3(AR)9 cells, DHT increases the growth by approximately 20% (Fig. 6*d*, lanes 1 *vs*. 7), and HAD (1 μM) and OAK (0.1 μM) significantly suppress the growth in the presence of DHT (lanes 7 *vs*. 8 and 9). In PC-3(AR)2, inconsistent with previous observation indicating that androgen induces apoptosis,[22] as well as AR-negative PC-3, DHT, HF, BC, and 3 DHEA derivatives only marginally affect cell proliferation (data not shown). We next determined whether HAD and OAK suppress PSA expression in prostate cancer cells. The Western blotting assay (Fig. 6*e*) shows that DHT increases endogenous PSA expression in LNCaP cells over mock treatment and that HAD (1 μM) and OAK (0.01–0.5 μM) decrease DHT-induced PSA expression. HAD and OAK slightly induce PSA expression in the absence of androgens, and it appears that the effects are greater than that of ADEK. The effects of steroid derivatives on AR protein expression were also examined, using NH27 polyclonal antibody for the AR.[17] None of the 3 inhibitory steroids affect AR expression in LNCaP cells (figure not shown). These results confirm our data of AR transcription and suggest that HAD and OAK can inhibit androgen-/AR-mediated prostate cancer progression.

TABLE I – SUMMARY OF AR LIGAND BINDING AFFINITY

Ligand	RBA value[1] in LNCaP	RBA value[1] in COS-1 with AR
DHT	100.0	100.0
HF	18.3	10.5
HAD	1.4	0.7
OAK	15.2	5.1
ADEK	6.7	3.0

[1]The RBA values were calculated from the constructed competitive binding curves as the ratio of concentration of unlabeled ligand and concentration of DHT required to inhibit [^{3}H]-R1881 binding by 50%. The RBA of DHT was set as 100.

Interruption of androgen binding to the AR by steroid derivatives

Clinically available antiandrogens are able to compete with androgens for binding to the AR. To determine whether HAD and OAK have this common feature of AR antagonists, the competitive androgen binding assay was performed. The affinity of ligands for the AR was assessed by incubating whole cell extracts of LNCaP or COS-1 with transfected WT AR with 1 nM [^{3}H]-R1881 in the presence of various concentrations (1–10,000 nM) of unlabeled DHT, HF, HAD, OAK or ADEK. As described previously,[16,25] the relative binding affinity (RBA) values were calculated from the constructed competitive binding curves as the ratio of concentration of unlabeled ligand and concentration of DHT required to inhibit [^{3}H]-R1881 binding by 50% (Table I). Competitive RBAs in LNCaP cells were DHT > HF > OAK > ADEK > HAD. Similar results were obtained in WT AR transfected COS-1 cells, although the RBAs are lower and binding of all the compounds in competition with [^{3}H]-R1881 was weaker. These results confirm that the steroid derivatives, especially OAK, compete significantly with androgens for AR binding.

Conclusion

We have tested whether steroid derivatives can block DHT-/Adiol-induced AR transactivation and have found 2 compounds, HAD and OAK, as potential antiandrogenic drugs to compete with androgens and block their action on both WT and mutant ARs. These compounds inhibited PSA expression and growth in prostate cancer cells. Their binding affinity to the AR was likely to be sufficient for the competition with androgen. Present results suggest that HAD at 1 μM or OAK at lower concentrations (50–100 nM) may control androgen-dependent prostate cancer progression. In addition, since the androgenic activity of HAD and OAK at potential therapeutic levels was very low and could not be induced by AR coactivators, there may be less possibility of inducing antiandrogen withdrawal syndrome if used for therapy in prostate cancer patients. More importantly, OAK was found to have only marginal estrogenic activity, which may provide potential quality of life benefits due to fewer unfavorable side effects. The effects of these steroid derivatives *in vivo* should be investigated, leading to the development of novel antiandrogenic drugs that block AR-mediated prostate cancer growth.

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