NCI, DCPC
Chemoprevention Branch and Agent Development Committee
CLINICAL DEVELOPMENT PLAN:
DEHYDROEPIANDROSTERONE
(DHEA)

DRUG IDENTIFICATION

CAS Registry No.: 53-43-0
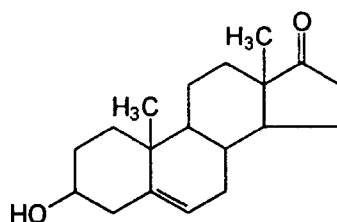
CAS Name (9CI): 3β-Hydroxyandrost-5-en-17-one
Synonyms: Prasterone
Androstenolone

Related compounds:
DHEA Analog 8354

Molecular Wt.: 288.4

Structure:

EXECUTIVE SUMMARY

Free DHEA and its sulfate conjugate, DHEA-S, are major steroid precursors secreted by the adrenal cortex. In peripheral tissues, they are metabolized to androstenedione, which can be converted to testosterone and estrogen; in fact, this is the source of much of the estrogen in postmenopausal women. Both retrospective and prospective epidemiologic studies suggest that serum DHEA or its urinary metabolites inversely correlate to human cancer risks. Positive associations between low levels of DHEA and cancer incidence have been reported for breast [e.g., 1–7], stomach [8], ovary [9], and bladder [10]. Only one of the breast cancer studies was a prospective study [2], all others were case-control studies. Associations between low levels of DHEA and other diseases have been sought, but not found, including osteoporosis [11], Alzheimer's disease [12] and benign breast disease [e.g., 13]. As described below, DHEA has shown promising preclinical chemopreventive activities against both spontaneous and chemically induced tumors [reviewed in 14]. There are some toxicities associated with high doses of DHEA in animals; analogs that bypass some or all of the toxicities of DHEA are under development, most notably the fluorinated analog 8354, which is scheduled for Phase I clinical trials. Although DHEA analog 8354 may have less toxicity in animal studies, the clinical formulation is still under development. For this reason, NCI is continuing the clinical evaluation of DHEA itself for chemoprevention of breast and prostate cancer.

The physiological role of DHEA as well as its molecular mechanism of action are unknown at this time. DHEA is a potent inhibitor of glucose-6-phosphate dehydrogenase (G6DPH), which catalyzes formation of extramitochondrial NAD(P)H and ribose-6-phosphate [15]. The chemopreventive ac-

tivity of DHEA may be due to a deficiency in these substances for DNA synthesis and cell proliferation [e.g., 16]. Depressed levels of NAD(P)H may also lead to decreased metabolism of carcinogens, due to reduced activity of mixed function oxidases, which also require NAD(P)H [reviewed in 17,18]. It has been noted that individuals with G6PDH deficiency have lower rates of cancer, and their lymphocytes are less efficient in metabolizing B(a)P [19,20]. Also, DHEA has been shown in vitro to block cell transformation by aflatoxin B_1 or DMBA [21]. Another activity, inhibition of protein isoprenylation, has been shown for both DHEA and DHEA-S [22–24]. Some proteins, including the ras oncogene, require post-translational isoprenylation to appropriately associate with the cell plasma membrane. Thus, this activity of DHEA and DHEA-S could help to slow cell growth. Other metabolic effects of DHEA include possible enhancement of cellular immunity, antiglucocorticoid activity [25], antiobesity, and antidiabetic effects [26], as well as inhibition of superoxide formation [27].

In NCI, Chemoprevention Branch-sponsored preclinical efficacy studies, DHEA has been effective in mammary gland, prostate and colon models of carcinogenesis. The published literature shows chemopreventive efficacy against development of mammary gland, skin, liver, thyroid, lung, colon and uterine cervix cancer, as well as lymphoma. These studies appear to support clinical development of DHEA.

There have been no Chemoprevention Branch-sponsored preclinical pharmacokinetics studies on this compound. Decreased body weight, increased liver weight, and estrogenic effects have been noted in six-month Chemoprevention Branch-sponsored toxicity studies, and these observations are supported in the literature.

DHEA and DHEA-S exist at high levels in the blood (0.01–0.02 µM and 5–7 µM, respectively) [25]. These levels rise from age 7 to a peak at about age 30 and then decline steadily with age. DHEA is converted primarily to DHEA-S in the adrenal gland, liver and kidney. This conversion is reversible, so that DHEA-S can be used to generate androstenedione. Conversion of DHEA to its downstream metabolites occurs primarily in liver and kidney [28]. The primary metabolites of DHEA are androsterone, etiocholanone (3α-hydroxy5β-androstan-17-one) and epiandrosterone [29]. These urinary metabolites, re-

ferred to as 17-ketosteroids, can be assayed in urine samples, but serum values of DHEA and DHEA-S are more accurate assessments of circulating adrenal androgens.

No Phase I clinical trials are planned with DHEA, as doses exceeding those planned for Phase II clinical trials have already been administered to humans. Doses ranging from 50–1,600 mg/day have been safely administered to men and women for up to one month, while doses of 50 or 200 mg/day have been administered for six months with only minor side effects. Protocols have been finalized for short-term Phase II trials in breast and prostate cancer patients. These trials involve a dose in the range of 200–300 mg/day administered during the period between diagnostic biopsy and surgical treatment, with the goal being to assess the effect of DHEA on intermediate biomarkers. For these studies, DHEA will be administered for two to four weeks. In addition, Phase II trials for borderline gammopathy and cervical dysplasia are being considered. A supply of formulated DHEA has been negotiated with Genelabs (Redwood City, CA) for the breast and prostate trials. If these trials are successful, longer term trials in patients at risk for breast (six months) or prostate (three years) cancer will be considered.

PRECLINICAL EFFICACY STUDIES

Evidence from both the published literature and from recent Chemoprevention Branch-sponsored studies shows the efficacy of DHEA in preclinical studies. Targets include both hormone-responsive (mammary gland, prostate, and uterine cervix) and nonhormoneresponsive (skin, colon, lung, bladder and thyroid) sites. These studies appear sufficient to support clinical development of DHEA.

In Chemoprevention Branch-contracted studies, evidence of DHEA efficacy was found in mammary gland, prostate and colon models of carcinogenesis. In MNU-induced rat mammary glands, DHEA at 1 or 2 g/kg diet (ca. 0.2 or 0.4 mmol/kg-bw/day), given one week prior to MNU and continued for the remainder of the experiment, reduced both the incidence and multiplicity of histologically confirmed carcinomas [30]. The 0.4 mmol/kg-bw/day dose produced significant weight loss relative to carcinogen controls (>10%). Given one week after MNU and continued for the duration of the experiment, this dose caused significant reduction of carcinoma incidence and multiplicity without weight loss. When the high dose

was given only one week prior to and one week following MNU, DHEA had no effect on tumor incidence or multiplicity, nor on body weight. A more recent study in MNU-induced rat mammary glands indicated efficacy for DHEA at lower doses of 120 or 600 mg/kg diet (ca. 0.02 or 0.1 mmol/kg-bw/day, respectively). The 0.1 mmol/kg-bw/day dose inhibited tumor incidence, multiplicity and number, while the 0.02 mmol/kg-bw/day dose inhibited only tumor multiplicity and number. Neither dose caused changes in body weight relative to carcinogen-treated controls. Even lower doses (5 or 24 mg/kg diet, ca. 0.0008 or 0.004 mmol/kg-bw/day) decreased tumor multiplicity by 30% and increased tumor latency, but did not affect final tumor incidence. Again, no effect on body weight was observed.

Combination studies in the MNU-induced rat mammary gland model indicate that DHEA alone at 1 or 2 g/kg diet (ca. 0.2 or 0.4 mmol/kg-bw/day) is as effective as DHEA plus 0.5 or 1 mmol 4-HPR/kg diet. These combination doses resulted in decreased body weight (p<0.05, but within 15% of carcinogen controls). A second combination study evaluated the effects of DHEA with carbenoxolone, tamoxifen citrate, or carbenoxolone plus tamoxifen citrate. DHEA was tested at doses of 400 or 800 mg/kg diet (ca. 0.07 or 0.14 mmol/kg-bw/day). At either of the relatively low doses, DHEA decreased both tumor incidence and multiplicity by more than 90%. As for the 4-HPR combination study, DHEA alone was as effective as any of the combinations tested. In these studies, no effects on body weights were noted [31].

In a Chemoprevention Branch-sponsored study of prostate tumors induced by cyproterone acetate, MNU, and testosterone propionate, 1 or 2 g DHEA/kg diet (ca. 0.2 or 0.4 mmol/kg-bw/day) was found to significantly decrease the incidence of macroscopic cancers in the hormone-promoted rat prostate without an effect on body weight gain. In the MAM acetate-induced mouse colon cancer model, Chemoprevention Branch-sponsored studies indicated that DHEA inhibited colon cancer at the lowest dose tested—0.15% DHEA in diet (ca. 0.7 mmol/kg-bw/day). However, this dose caused a significant decrease in body weight (p<0.05).

Reports from the published literature also support the chemopreventive activity of DHEA in rat mammary glands [e.g., 32,33], as well as mouse skin

papillomas and carcinomas induced in CD-1 mouse skin by DMBA/TPA [34,35], spontaneous Leydig cell hyperplasia and tumors (tumor type not specified) in aging rats [36], and GGT-positive liver foci induced by DEN and promoted by phenobarbital, partial hepatectomy and acetylaminofluorene [37]. DHEA has been shown to be effective against AOM-induced aberrant crypt foci in rat colon when provided prior to initiation [38]. Efficacy has also been shown against DMBA-or urethane-induced lung adenomas in A/J mice [39] and against uterine cervix squamous cell carcinomas induced by 3-methylcholanthrene in mice [40]. DHEA reduced the number of thyroid adenomas induced by dihydroxy-di-n-propylnitrosamine in rats [41]. Finally, in a p53 knock-out mouse model, DHEA significantly decreased lymphoma incidence and increased survival when administered at 0.3% in the diet (ca. 1.3 mmol/kg-bw/day) [42].

PRECLINICAL SAFETY STUDIES

No 90-day Chemoprevention Branch-sponsored studies have been performed to date. One six-month toxicity study looked at the hormonal effects of DHEA and DHEA analog 8354 in rats. Further preclinical testing in another species (e.g., dog) may be needed for performance of Phase II clinical trials greater than six months duration.

Safety: Published reports indicate that DHEA has toxic effects in the liver [e.g., 43,44], with one study indicating DHEA is a rat liver carcinogen when administered at 0.45% in the diet (ca. 0.8 mmol/kg-bw/day) for 84 weeks [45]. Long-term studies are necessary to determine if chronic DHEA administration at lower doses causes hepatocarcinogenesis. DHEA has been a peroxisome proliferator at ca. 0.35 or 0.8 mmol/kg-bw/day in rats (administered in the diet) and at 1 mmol/kg-bw/day in rats and mice (administered intragastrically) [e.g., 46–48]. Peroxisome proliferators are known carcinogens, although the exact mechanism by which they cause cancer is under investigation [e.g., 49,50]. Most peroxisome proliferators appear to have minimal effects in primates; no data support their carcinogenicity in primates. Unpublished data suggest that the effect of DHEA on peroxisomal enzymes is dose-dependent. At low doses that still possess chemopreventive ac-

tivity, such as 125 ppm in the diet (ca. 0.02 mmol/kg-bw/day), effects on peroxisomal enzymes are minimal. A dose of 600 ppm (ca. 0.1 mmol/kg-bw/day) produces only 25% the induction of a 2,000 ppm dose (ca. 0.35 mmol/kg-bw/day) [51]. DHEA also produced hepatomegaly and increased the number of basophilic foci induced by dihydroxy-di-n-propylnitrosamine [e.g., 52,53].

Chronic administration of DHEA in the diet has also reduced body weight without reducing food intake, although this problem can be negated with lower dosing [14]. For example, chemopreventive efficacy in rat mammary glands was observed in a Chemoprevention Branch-sponsored study at 600 mg/kg diet (ca. 0.1 mmol/kg-bw/day), with no effect on body weight relative to carcinogen-treated controls. Thus, it may be possible to find effective doses that do not alter body weight.

Chemoprevention Branch-contracted studies evaluated the hormonal effects of both DHEA and analog 8354. Ovariectomized female rats fed either agent at 2 g/kg (ca. 0.35 mmol/kg-bw/day) for six months had greater uterine weights than ovariectomized animals fed basal diet. The DHEA analog 8354-fed group had two-fold higher uterine weights than DHEA-fed rats, suggesting a somewhat greater estrogenic effect. Liver weights were increased in groups treated with either agent compared with intact controls; however, the increase was statistically significant only for DHEA.

The study also evaluated androgenic effects of both agents in castrated male rats. After administration of 400 mg/kg diet (ca. 0.07 mmol/kg-bw/day) for six months, castrated rats receiving DHEA had seminal vesicle weights greater than castrated rats receiving basal diet, while those treated with DHEA analog 8354 had seminal vesicle weights comparable to controls. Neither agent produced changes in prostate weights.

Preclinical studies published in the literature have highlighted the major toxicities of this compound, namely liver (discussed above) and steroidal effects (uterine enlargement, as well as ovarian tumors in susceptible mice [54–57]). Another target tissue is the pancreas, where DHEA has promoted the number of glutathione S-transferase (A-form)-positive lesions induced by AOM in the hamster [58]. A metabolite of DHEA, androstenedione, stimulated the growth of established rat mammary gland tumors initiated by DMBA, while not affecting uterine weight [59].

Many of the toxicities associated with DHEA appear to occur at higher doses, so it may be possible to find effective doses which minimize the side effects.

DHEA is an abortifacient in pregnant rats when administered between days 2 and 19 of gestation at a dose of 2 mg/kg-bw/day [60]. Virilization of the external genitalia of fetal female rats has also been reported [61], as well as precocious maturation in immature female rats [57].

In vitro studies have shown that DHEA can bind to a mutant human androgen receptor, and that binding of DHEA to mutant receptors activates transcription more efficiently than binding to wild type receptors. The mutant androgen receptor was cloned from a poorly differentiated, endocrine therapy-resistant prostatic carcinoma, and it differed from the wild-type receptor by only one amino acid [62]. Such an observation underscores the lack of understanding of DHEA's mechanism of action, and demonstrates a mechanism that allows DHEA to directly interact with hormone signalling systems. Depending upon the tissue, such interactions could be beneficial or harmful.

ADME: The combination study of DHEA plus carbenoxolone, tamoxifen citrate or carbenoxolone plus tamoxifen citrate in the MNU-induced mammary gland study described above included analysis of the serum concentrations for each of the chemicals given in combination. There were no measurements of effects of DHEA alone on serum levels; all measurements were made on animals dosed with two or more chemicals. The results indicated that oral administration of DHEA did raise the serum levels of DHEA over time and that the levels remained elevated with continued dosing. The 0.14 mmol/kg-bw/day dose of DHEA produced higher serum levels of DHEA than the 0.07 mmol/kg-bw/day dose (70.8 ± 10.3 µg/ml vs. 64.4 ± 20.3 µg/ml after 25 weeks of administration), but the differences in serum concentration were not significant.

CLINICAL SAFETY: PHASE I STUDIES

No Chemoprevention Branch-sponsored Phase I chemoprevention trials are underway nor are any planned, as DHEA has been used in the clinical setting. Results of Phase I trials from the published literature using physiological and pharmacological doses of DHEA in various adult populations are briefly summarized below. None of these trials focused on chemoprevention issues, but the studies do

provide evidence of safety at the 200–300 mg/day dose planned for one-month Phase II trials in breast and prostate cancer patients.

Drug Effect Measurement: The published studies used a variety of drug effect measurements. Serum levels of androgens, estrogens and cholesterol, general feelings of well being, insulin resistance, percent body fat, and changes in IGF-I bioavailability were common measurements made in the Phase I studies. The studies demonstrate that serum levels of DHEA reflect the dose, although none of the studies produced conclusive dose-response curves. Commercial radioimmunoassay (RIA) kits are available to measure serum DHEA concentrations. Concurrent measurements of DHEA-S, also easily made with commercial kits, can provide evidence that DHEA is available for biotransformation.

Safety: A wide variety of Phase I trials have appeared in the published literature. Neither physiologic nor pharmacological doses produced severe side effects in men or women. Single-dose trials of 150 or 300 mg DHEA/day (*ca.* 0.008 or 0.016 mmol/kg-bw/day) were conducted in postmenopausal women [63]. An open-label, noncontrolled six-month trial with a dose of 200 mg/day (*ca.* 0.011 mmol/kg-bw/day) was conducted in female lupus patients [64]. Randomized, double-blind, placebo-controlled trials (some with crossover) have been conducted in aging men and women with a dose of 50 mg/day (*ca.* 0.002 mmol/kg-bw/day) for durations of two to six months [65–67]. Several one-month trials, conducted in normal and obese men and postmenopausal women, have utilized pharmacological doses of DHEA (1,600 mg/day, *ca.* 0.08 mmol/kg-bw/day) [68–71]. Only one trial reported any changes in clinical laboratory values (decreased serum low density lipid cholesterol levels [68]). The primary side effect noted in the published studies was hirsutism among women [64,65,71]. The dose of 50 mg/day caused supraphysiological levels of testosterone in postmenopausal women [67]. Testosterone, androstenedione, and dihydrotestosterone levels were unchanged in aging men at 50 mg/day [65]. The consensus from these studies is that orally administered DHEA is well tolerated by men and women at doses up to *ca.* 0.08 mmol/kg-bw/day for one month. At lower doses (*ca.* 0.002 mmol/kg-bw/day), DHEA is well tolerated for six months.

One open-label, dose-escalated trial of DHEA was conducted in HIV-positive men. The doses tested were 750, 1,500 and 2,250 mg/day (*ca.* 0.04 to 0.11 mmol/kg-bw/day) for four months. These doses were well tolerated, with no dose limiting side effects noted [72].

ADME: The above published studies generally agree that orally administered DHEA is rapidly absorbed and biotransformed to DHEA-S and androgenic (*e.g.*, androstenedione, testosterone or dihydrotestosterone, particularly in women) and estrogenic metabolites at doses ranging from 50 to 1,600 mg/day (*ca.* 0.002 to 0.08 mmol/kg-bw/day). Serum levels of DHEA rise rapidly (within 180 minutes), remain elevated for the duration of exposure (up to six months), and decline to baseline levels within one week following withdrawal of the compound [64,71]. In some of the studies in women, DHEA serum levels peaked before DHEA administration was terminated [64,71]. It remains to be determined if the decline reflects decreased absorption, increased clearance, or both.

Several published studies have examined the metabolic clearance of DHEA utilizing both single injection and constant infusion methods. One study of normal subjects indicated a rapid metabolic clearance rate (Cl_p) of 1,866±144 l/24 hours for men, and 1,901±87 l/24 hours in women, with the values determined using a two-compartment model. In this study, plasma levels of DHEA were 8.5±1.0 ng/ml for men, and 8.8±1.0 ng/ml for women. This study observed no sex-linked difference in binding of DHEA to plasma proteins, which is reflected in the lack of sex difference in the maximal clearance rate. The authors suggest that the rapid clearance and large volume of distribution (V_1=38.5±6.0 l, V_2=30.4±7.3 l for men; V_1=33.7±5 l, V_2=27.5±9.9 l for women) of DHEA relative to structurally related steroids may stem from the fact that DHEA does not bind the sex hormone-binding globulin, as other androgens do [73]. Another study in women (8 mg/hour by constant infusion) found the Cl_p nearly identical to the value above, with a $t_{1/2}$ for DHEA of 44.2±8.4 minutes [74]. Thus, DHEA is rapidly metabolized, primarily to DHEA-S, and also to androgenic metabolites (with women showing a greater conversion of DHEA to androgenic metabolites than men) [73]. One study demonstrated conversion of DHEA to estradiol in pregnant women at term [74]. None of the studies examined urine levels of DHEA metabolites in conjunction with the serum metabolites.

The study of HIV-infected men described above

suggested that there may be two populations with different bioavailability of orally administered DHEA, as there was no correlation of the doses tested to the serum levels of DHEA achieved. Some of the men achieved serum levels far greater than others at the same dose. For example, C_{max} for the 1,500 mg/day dose ranged from 13 to 145 ng DHEA/dl, while the C_{max} for the 2,250 mg/day dose ranged from 18 to 198 ng DHEA/dl. A larger trial (this trial only comprised 31 subjects) designed to examine this issue would be needed to confirm this observation. Urinary 17-ketosteroids increased 1.4-and 2.7-fold in the medium and high-dose groups, respectively, relative to the low-dose group [72].

Metabolic clearance rates for DHEA-S have been reported in several publications, with a Cl_p value around 13 1/24 hours for both men and women, demonstrating a far greater stability relative to DHEA, consistent with the higher steady state levels of DHEA-S in serum [e.g., 75–78].

CLINICAL EFFICACY: PHASE II/III STUDIES

The Chemoprevention Branch has funded short-term Phase II trials in breast and prostate cancer patients during the interval between diagnostic biopsy and surgery in order to assess the effect of DHEA on intermediate biomarkers. Protocols are currently being finalized for these short-term studies. The trial in breast cancer patients will be conducted by Drs. Marie Pennanen and Jose M. Esteban at the Georgetown University Medical Center. Patients with intraductal and early invasive breast carcinoma will be given an oral formulation of DHEA at 200–300 mg/day (ca. 0.011–0.016 mmol/kg-bw/day) during the period between diagnostic biopsy and surgery (two to four weeks). Endpoints for this trial include nuclear morphometry, DNA ploidy, erb-2 expression, p53 expression, cellular adhesion molecules (CD44), vascular proliferation, EGFR, proliferation markers (PCNA, Ki-67, cyclin D1), and FISH analysis of chromosomes 1, 7, 11 and 17.

The trial in prostate cancer patients will be conducted by Dr. Kenneth J. Pienta at the University of Michigan Comprehensive Cancer Center. DHEA will be administered at 200–300 mg/day (ca. 0.011–0.016 mmol/kg-bw/day) during the period between diagnostic biopsy and radical prostatectomy (four weeks). Endpoints for this trial include density and grade of PIN lesions, proliferation index, DNA ploidy, p53 expression, nuclear morphometry, fractal

lesion analysis, PC-1 expression, and chromosome 8p loss.

The chemopreventive efficacy of DHEA for these tissues is suggested by preclinical efficacy, as well as epidemiological data. As previously mentioned, serum levels of DHEA decline with age, while the incidence of both prostate and breast cancer increases with age [79]. In addition, subnormal values of DHEA and DHEA-S are observed in women with breast cancer [e.g., 7]. In a prospective study, 24-hour urinary steroid levels were obtained from women on the island of Guernsey. The excretion of DHEA metabolites was lower in women who developed breast tumors over the following nine years [2]. Urinary DHEA levels were significantly lower in women who developed ovarian cancer over 130 months [9].

In addition, Phase II trials are being considered for monoclonal gammopathy, a precursor to multiple myeloma, and cervical dysplasia. Monoclonal gammopathy is associated with older populations; a fraction of these patients appears to progress to multiple myeloma. Although the exact mechanism of disease progression is unclear, increased levels of the cytokine IL-6 appear to be important in the process [80]. DHEA may be able to alter the ratio of T-helper (Th) cell subsets (increasing Th1 relative to Th2), thereby causing a decrease in Th2-related cytokines (IL-4, IL-5, IL-6 and IL-10) and boosting cellular immunity [81]. Feeding DHEA to aged laboratory animals leads to a decrease of age-related increases of IL-6 production [82]. Thus, administration of DHEA may help to lower circulating levels of IL-6 and prove beneficial to patients with monoclonal gammopathy.

The cervical dysplasia trial is being considered due to efficacy in mice treated with 3-methylcholanthrene. In addition, numerous studies indicate that HPV and the activated ras oncogene are able to transform primary cells [e.g., 83,84]. This process appears to be enhanced by the presence of glucocorticoids [e.g., 84,85]. Since DHEA blocks protein isoprenylation (which may prevent the ras protein from functioning) and has anti-glucocorticoid effects [reviewed in 25], it may be efficacious against cervical dysplasia.

PHARMACODYNAMICS

The lowest effective preclinical chemopreventive dose observed to date is 0.02 mmol/kg-bw/day in the MNU-induced rat mammary carcinogenesis model. This dose reduced tumor multiplicity and number. A

dose of 0.1 mmol/kg-bw/day inhibited tumor incidence, multiplicity and number in the same model. The lowest carcinogenic dose reported, 0.8 mmol/kg-bw/day in rats, is 8–40 times higher than the chemopreventive dose in rats. Administration to humans revealed that 0.002 mmol/kg-bw/day was sufficient to raise serum levels of DHEA in aging people to those similar to younger subjects without serious side effects [65]. In normal subjects, doses up to 0.08 mmol/kg-bw/day have been administered without side effects for one month [68,71]; HIV-positive men have tolerated doses up to 0.11 mmol/kg-bw/day during a four month dose-escalation trial [72]. Preclinical toxicity testing may provide a NOEL to establish the margin of safety for long-term administration of DHEA. The existing data suggest the 200–300 mg/day dose (0.011– 0.016 mmol/kg-bw/day) planned for the short-term trials is a safe chemopreventive dose for up to four weeks.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

For clinical development of DHEA, a reliable, sensitive and specific drug effect measurement for DHEA should be identified. One choice for evaluation is measurement of G6PDH activity in lymphocytes.

Commercial RIA kits are available to measure DHEA or DHEA-S in the serum. For example, Wein Laboratories (Succasunna, NJ) provides kits to measure serum DHEA and DHEA-S, while Pantex Ltd. (Santa Monica, CA) provides kits for measuring serum DHEA-S. Several of the published human studies have used these kits and report coefficients of variation under 10%. These tests can be used to verify absorption and biotransformation of DHEA.

Safety Issues

The major toxicological issues are the potential for liver and hormone-related effects in humans. Further assessments of the hepatocarcinogenicity of this compound may be prudent for long-term trials. Hepatocarcinogenicity has only been shown in the rat, so the possibility remains that the rat is particularly susceptible. As with tamoxifen citrate, estrogenic effects such as uterine and ovarian proliferation are a concern. Higher levels of DHEA have been reported in breast tumor tissues relative to normal breast tissue

[86]. DHEA may have different effects (e.g., androgenic versus estrogenic) depending upon the hormonal status of the individual receiving the compound (e.g., premenopausal versus postmenopausal), as DHEA can be converted to androgens or estrogens in a variety of tissues [87]; levels of hormones in particular tissues within an individual could determine which metabolites are formed. These androgenic versus estrogenic effects may also influence any toxic or carcinogenic effects of the compound. Androgen and estrogen levels should be monitored whenever serum DHEA measurements are made in order to anticipate problems arising from excess hormone production. FSH and LH could also be monitored to determine if DHEA is having an effect on the pituitary axis.

Pharmacodynamics Issues

Determination of the margin of safety for DHEA is a goal of Phase II clinical trials. Humans have received doses within the range of those that produce chemopreventive effects in rodents, and those doses appear to be well tolerated.

Regulatory Issues

The full complement of preclinical toxicity studies has not been performed by the Chemoprevention Branch. However, the compound has been given to humans in trials focused on other clinical indications. Longer Phase II trials (greater than six months) may require preclinical toxicity studies in another species. Additional data from Phase I trials may also be needed to establish dosing schedules for long-term Phase II trials (e.g., to evaluate the possibility of intermittent dosing).

Intermediate Biomarker Issues

For the Phase II trials initiated in breast and prostate cancer patients, intermediate biomarkers of interest include nuclear pleomorphism index, DNA ploidy, oncogene expression, and cell proliferation indices.

DHEA has been shown to be effective against AOM-induced aberrant crypt foci in rat colon, DMBA/TPA-induced skin papillomas in mice, and DMBA or urethane-induced lung adenomas in mice. DHEA has also been shown to inhibit superoxide formation, decrease carcinogen binding to DNA, decrease DNA synthesis and inhibit protein isoprenylation.

Supply and Formulation Issues

Negotiations for an oral formulation of DHEA have been completed for the breast and prostate trials with Genelabs (Redwood City, CA). Additional collaborations may be pursued for trials at other targets.

Clinical Studies Issues

Phase I trials published in the literature have established safety for orally administered DHEA in adults. These trials have demonstrated that oral DHEA is absorbed, evidenced by increased serum levels of DHEA, and available for biotransformation, evidenced by increased serum DHEA-S. Phase II trials for prostate and breast cancer patients are in progress. These targets were selected due to efficacy in animal models for these diseases. DHEA is an interesting compound for hormonally sensitive targets, as it is metabolized into compounds that can be converted to androgens or estrogens. Therefore, DHEA may be able to correct imbalances in androgen and estrogen levels that may underlie cellular proliferation, although evidence for such a mechanism is scant. The nonsteroidal activities of DHEA, such as inhibition of G6PDH and protein isoprenylation, may be the mechanisms behind the chemopreventive activities of DHEA.

The trials currently in progress involve patients between diagnostic biopsy and surgery, with a major aspect of these trials being dose-titration against nuclear pleomorphism index, DNA ploidy, oncogene expression, and cell proliferation indices as potential surrogate biomarkers for cancer incidence. Phase II trials for borderline gammopathy and cervical dysplasia are being considered. These trials will be short-term studies focused on identifying pertinent biomarkers. Age stratification of the results will be considered, as DHEA levels decline with age.

If these short-term studies are successful, longer trials in patients at risk for breast or prostate cancer will be considered. For the high-risk breast cancer cohort, a six-month study in patients with fine-needle aspirates showing dysplasia or hyperplasia and other biomarker abnormalities is planned. For the at-risk prostate cancer cohort, a three year study in men with PIN is being considered. Due to concerns that DHEA may be androgenic in the prostate (*i.e.*, DHEA may be converted to dihydroxytestosterone in the prostate [87]), the dose of DHEA may be reduced to 50 mg/day, or DHEA may tested in combination with a

5α-reductase inhibitor. An additional alternative would be to use DHEA analog 8354 (which is currently undergoing formulation) instead of DHEA in prostate trials, as the analog has fewer androgenic activities.

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Table I. Clinical Trials of DHEA Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers)					
NO1-CN-65002 Phase II Clinical Trial of DHEA in Patients with Prostate Cancer (Dr. Kenneth J. Pienta, University of Michigan Comprehensive Cancer Center) 10/95-	Prostate	Patients with prostate cancer (50–70 years) undergoing radical prostatectomy 100 subjects (50/arm)	300 mg/day po for 28 days between diagnostic biopsy and surgery	Efficacy: Nuclear morphometry, proliferation index, PIN, DNA ploidy, p53 expression, bcl-2 expression,fractal lesion analysis, PC-1 expression, chromosome 8p loss Risk factors: Serum PSA Safety: Serum DHEA and DHEA-S	Evaluation of intermediate biomarkers
NO1-CN-65001 Phase II Clinical Trial of DHEA in Breast Neoplasia. Administration During the Period Between Diagnostic Core Biopsy and Definitive Surgery (Drs. Marie Pennanen and Jose M. Esteban, Georgetown University Medical Center) 10/95-	Breast	Patients with intra-ductal and early invasive breast carcinoma scheduled for surgery 100 subjects (50/arm)	300 mg/day po for 2–4 wk between diag-nostic biopsy and surgery	Efficacy: Nuclear morphometry, DNA ploidys, erbB-2 ex-pression, p53 expression, cellular adhesion molecules (CD44), vascular proliferation, EGFR, proliferation markers (PCNA, Ki-67, cyclin D1), FISH analysis of chromosomes 1, 7, 11 and 17 Safety: Serum DHEA and DHEA-S	Evaluation of intermediate biomarkers

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Table I. Clinical Trials of DHEA Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose titration, efficacy, intermediate biomarkers) *(continued)*					
Planned Study Phase II Clinical Trial of DHEA in Monoclonal Gammopathy of Undetermined Significance (MGUS) and Monoclonal Gammopathy of Borderline Significance (MGBS)	Hemato-poietic	Males and females >40 yr with monoclonal gammo-pathy >2 g/dl, bone marrow plasma-cytosis not >30%, without multiple myeloma or B-cell neoplasm 100 subjects (50/arm)	200 mg/day po for 6 mo	Efficacy: Nuclear morphometry, DNA ploidy, Th1/Th2 ratios in peripheral blood, IL-6 serum levels, other cytokine serum levels (*e.g.*, IL-1, IL-10, IL-12), cellular adhesion molecules (*e.g.*, CD44), oncogene and/or tumor suppressor expression (*e.g.*, *c-myc*, p53), proliferation markers (*e.g.*, Ki-67), G6PDH activity Safety: Serum DHEA and DHEA-S	Evaluation of intermediate biomarkers
Planned Study Phase II Clinical Trial of DHEA in Patients with Cervical Intraepithelial Neoplasia (CIN) Grade 3. Histological Reduction of CIN 3 and Modulation of Surrogate Endpoint Biomarkers	Cervix	Patients with CIN III 100 subjects (50/arm)	200 mg/day po for 6 mo between diagnostic biopsy and surgery	Efficacy: Nuclear morphometry, CIN III regression, DNA ploidy, proliferation markers (*e.g.*, Ki-67), EGFR, oncogene expres-sion (*e.g.*, *ras*) Safety: Serum DHEA and DHEA-S	Evaluation of intermediate biomarkers

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DHEA DEVELOPMENT STATUS

Task Name	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
PRECLINICAL EFFICACY	■	■	■	■	■	■	■	■	■	■	■	■	■
PRECLINICAL PHARMACOLOGY										■	■	■	
PRECLINICAL TOXICOLOGY											■		
CLINICAL, PHASE II												■	■
COMBINATIONS													
PRECLINICAL EFFICACY			■	■	■		■	■	■				
PRECLINICAL PHARMACOLOGY							■	■	■				

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