NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

DHEA ANALOG 8354

DRUG IDENTIFICATION

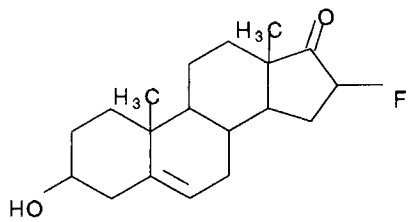
CAS Registry No.: 112859-71-9

CAS Name (9CI): 16α-Fluoro-5-androsten-17-one

Synonyms: Fluasterone

Related Compounds:
Dehydroepiandrosterone (DHEA)

Structure:



EXECUTIVE SUMMARY

Free and conjugated DHEA are major steroid precursors secreted by the adrenal cortex. In peripheral tissues, they are metabolized to testosterone and estrogen; in fact, this is the source of much of the estrogen in post-menopausal women. There is some epidemiological evidence that circulating DHEA correlates inversely with cancer risk [reviewed in 1]. Although DHEA appeared promising in preclinical chemopreventive efficacy studies [reviewed in 2], it had significant liver (hepatomegaly, peroxisome proliferation, basophilic foci) and steroidal toxicity (uterine enlargement, increased seminal vesicle weight) [3,4]. To decrease these effects, Dr. A.G. Schwartz and co-workers [5] synthesized the fluorinated derivative, DHEA analog 8354. Both compounds are potent inhibitors of glucose-6-phosphate dehydrogenase (G6PDH), which catalyzes formation of extramitochondrial NAD(P)H and ribose-6-phosphate. The chemopreventive activity of DHEA and DHEA analog 8354 may be due to a lack of these substances for DNA synthesis and proliferation. In fact, the addition of the four deoxyribonucleosides to the drinking water of mice during TPA promotion completely reverses DHEA analog 8354-induced inhibition of skin tumors [6]. Alternatively, reduction in the NAD(P)H pool may reduce the activity of mixed function oxidases and the activation of certain carcinogens. 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 [2]. In addition, DHEA analog 8354 inhibits TPA-induced prostaglandin synthesis [7] and may have some antiviral properties [8]. Since DHEA analog 8354 appears to retain [9] or surpass [10] the cancer inhibitory activity of the parent without the toxicity, it is being considered for further development as a cancer chemopreventive drug.

Since DHEA analog 8354 is a more potent inhibitor of adrenal G6PDH than DHEA, it has greater chemopreventive efficacy in some pre-

clinical studies. The analog is more effective against mouse skin tumorigenesis than the parent, with concomitant reduction in carcinogen-DNA binding and [^{3}H]-thymidine incorporation. Both DHEA and the fluorinated analog were equally effective against mammary gland carcinogenesis in the rat, and both agents inhibited colon cancer at the lowest doses tested. Although the analog has not been tested in preclinical prostate cancer models, DHEA inhibited macroscopic cancer incidence in the rat. These studies appear to be sufficient to support clinical development of DHEA analog 8354.

In preclinical pharmacokinetics studies contracted by the CB, the agent appears to have a short plasma $t_{1/2}$ and does not accumulate in rats or dogs. In toxicity studies, adverse effects were hypocholesterolemia and decreased weight gain. The increased liver weight observed in DHEA-treated animals was not significant with DHEA analog 8354. The data on estrogenic effects of DHEA analog 8354 varied with the assay used; however, no androgenic effects were reported.

Epidemiological data and retrospective and prospective studies suggest that DHEA or its urinary metabolites inversely correlate with human cancer risk. Since DHEA and DHEA analog 8354 have similar activities related to chemopreventive efficacy, pharmacological doses of DHEA analog 8354 may inhibit carcinogenesis. No human safety and pharmacokinetics data are available from the literature, and clinical studies have not been sponsored by the CB. The CB is considering three short-term Phase II trials in breast and prostate cohorts for 1995. Negotiations are underway with Research Corporation Technology to supply drug to the CB. Because of absorption problems in preclinical pharmacokinetic studies, DHEA analog 8354 needs to be reformulated.

PRECLINICAL EFFICACY STUDIES

Since DHEA analog 8354 is a more potent inhibitor of adrenal G6PDH than DHEA, it surpasses the chemopreventive efficacy of DHEA in some preclinical studies. The analog is a more potent inhibitor of mouse skin tumorigenesis than the parent, with concomitant reduction in DMBA-DNA binding and [^{3}H]-thymidine incorporation. Both DHEA and the fluorinated analog were effective in mammary gland and colon models of carcinogenesis. These studies appear sufficient to support clinical development of DHEA analog 8354.

In CB-contracted studies, both agents inhibited colon cancer at the lowest doses tested—100 ppm DHEA analog 8354 (*ca.* 0.02 mmol/kg-bw/day) in the AOM-induced rat model [11] and 0.15% DHEA in diet (*ca.* 0.6 mmol/kg-bw/day) in the MAM-acetate-induced mouse model. In MNU-induced rat mammary glands, DHEA analog 8354 (1 g/kg diet, or *ca.* 0.2 mmol/kg-bw/day) reduced the multiplicity of histologically confirmed tumors; however, the same dose of DHEA not only reduced tumor multiplicity, but inhibited the incidence of palpable tumors as well [9]. Although DHEA analog 8354 has not been tested in preclinical models of prostate cancer, 1 and 2 g DHEA/kg diet (*ca.* 0.2 mmol/kg-bw/day) was recently found to significantly decrease the incidence of macroscopic cancers in the rat prostate without an effect on body weight gain.

In published studies, dietary DHEA analog 8354 (2 g/kg diet, or *ca.* 0.8 mmol/kg-bw/day) was effective against DMBA-induced/TPA-promoted (top) mouse skin papillomas when given during either initiation or promotion [10]. When administered from one week before to one week after DMBA initiation, DHEA analog 8354 reduced tumor multiplicity by 67%; at the same dose, DHEA had no effect. Inhibition (67%) of the binding of [^{3}H]-DMBA to mouse epidermal DNA by the analog (200 mg/kg-bw/day, or 0.7 mmol/kg-bw, po) was demonstrated in a separate experiment [5]. DHEA analog 8354 was equally effective (60% inhibition) when administered beginning one week after DMBA initiation; DHEA inhibited tumor multiplicity by only 36% [10]. During these studies, however, both steroids caused a significant decrease in weight gain compared with controls. A second study was performed to determine if DHEA analog 8354 had tumor inhibitory activity distinct from weight reduction. In the same two-stage mouse skin tumorigenesis model, 200 μg (0.0007 mmol) of the agent was applied topically (3x/week for 64 days) one hour before each TPA application [6]. Since tumor multiplicity decreased 60% without any effect on body weight gain, DHEA analog 8354 had a direct effect on the epidermis. Interestingly, food restriction and DHEA analog 8354 appear to act by the same mechanism—inhibition of G6PDH [12].

PRECLINICAL SAFETY STUDIES

Based on dietary and intubation studies, the agent appears to have a short plasma $t_{1/2}$ and does

not accumulate in rats or dogs. In toxicity studies, adverse effects were hypocholesterolemia and decreased weight gain. The liver changes observed in DHEA-treated animals were not seen with DHEA analog 8354. The data on estrogenic effects of DHEA analog 8354 varied with the assay used; however, no androgenic effects were reported.

ADME Pharmacokinetics studies on DHEA analog 8354 in two species have been completed by the CB. In male and female Sprague-Dawley rats, single doses of DHEA analog 8354 suspended in oil (ig) did not show linear pharmacokinetics. At 200 mg/kg-bw (0.7 mmol/kg-bw), C_{max}=0.8 μg/ml, terminal $t_{½}$=2.3 hr, t_{max}=3.6 hr, and AUC_{0-32}= 4.5 μg/ml·hr; at 1,000 mg/kg-bw (3.3 mmol/kg-bw), C_{max}=0.6 μg/ml, terminal $t_{½}$=4.8 hr, t_{max}= 5.4 hr, and AUC_{0-32}=3.9 μg/ml·hr. The lack of dose dependence for AUC and C_{max} values may have resulted from the properties of the drug suspension. Since the same volume was administered at both levels, the suspension for the higher dose was more viscous and did not appear to be absorbed as well.

In multidose studies in the rat, the mean weekly plasma levels were approximately 0.01 μg/ml at 0.1% DHEA analog 8354 in the diet and 0.01–0.02 μg/ml at 0.2% diet for four weeks. The agent appeared to be rapidly eliminated, with no potential for accumulation.

In male and female Beagle dogs, the pharmacokinetics of single doses of DHEA analog 8354 (ig) were also not dose-dependent. C_{max} increased only two-fold with a five-fold increase in dose. For example, at 100 mg/kg-bw (0.3 mmol/kg-bw), C_{max}=1.5 μg/ml, terminal $t_{½}$=2.1 hr, t_{max}=1.6 hr, and AUC_{0-72}=7.5 μg/ml·hr; at 50 mg/kg-bw (0.2 mmol/kg-bw), C_{max}=2.3 μg/ml, terminal $t_{½}$= 4.3 hr, t_{max}=3.7 hr, and AUC_{0-72}=24.6 μg/ml·hr. After dosing (ig) for 14 days, the plasma levels were lower than after the first dose, suggesting altered elimination, metabolism or distribution.

Safety CB-contracted 90-day subchronic studies in rats (up to 1 g/kg-bw/day, or 3.3 mmol/kg-bw/day) and dogs (up to 250 mg/kg-bw/day, or 0.8 mmol/kg-bw/day) have established a NOEL of 250 mg/kg-bw/day (0.8 mmol/kg-bw/day) in both species [13]. No histopathological effects were observed. At the highest doses tested in male rats, toxic effects included a dose-related decrease in weight gain and hypocholesterolemia.

In a published subchronic study, dietary treatment of female mice with 0.2% DHEA analog 8354 (*ca.* 0.8 mmol/kg-bw/day) and TPA (top) for two weeks significantly reduced body weight (13%) compared with TPA-treated controls; however, the reduction (8%) produced by 0.1% in the diet (*ca.* 0.4 mmol/kg-bw/day) was not significant [7]. Food consumption decreased 34%. In contrast, DHEA had no effect on body weight or food consumption at the same doses.

Additional CB-contracted studies evaluated the hormonal effects of both DHEA and the analog. Ovariectomized female rats fed either agent at 2 g/kg diet (*ca.* 0.3 mmol/kg-bw/day) for six months had significantly lower uterine weights than intact animals on basal diet or ovariectomized animals implanted with estrogen. The DHEA analog 8354-fed group, however, 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 to 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 either agent had seminal vesicle weights equal to castrated rats receiving basal diet; the seminal vesicle weights of these three groups (6–10 mg/100 g-bw) were much less than in intact rats (161 mg/100 g-bw). The same lack of androgenic effect was observed in prostate weights.

In a published study on the estrogenic effects of DHEA analog 8354, injection (sc) of 60 mg/kg-bw (0.2 mmol/kg-bw) for three days did not increase uterine weight in sexually immature female mice; DHEA increased this parameter two-fold [5]. In the same study, an assay of androgenic effects in castrated male mice found no increase in seminal vesicle weights after 11 days of DHEA analog 8354 treatment at the same dose; however, DHEA increased this measurement 2.5-fold. Also, dietary DHEA analog 8354 (0.25% diet, or *ca.* 1.1 mmol/kg-bw/day) did not increase liver weight or catalase activity after three weeks of treatment, although DHEA had significant effects.

CLINICAL SAFETY: PHASE I STUDIES

No clinical safety and pharmacokinetics data are available from the literature. In the general population, normal plasma levels of DHEA and DHEA sulfate are low until the age of 7, peak at age 30, and then decrease steadily throughout life [reviewed in 1].

CLINICAL EFFICACY: PHASE II/III STUDIES

Three Phase II chemoprevention trials in hormone-responsive tissues are under consideration by the CB for 1995. Two trials involve treatment of cancer patients in the period between diagnostic biopsy and surgery—the individual trials address breast and prostate cancer. The prostate trial will also accrue observation-only incidental cancer patients and PIN patients. A second prostate trial will evaluate DHEA analog 8354 in men at high risk for prostate cancer. The chemopreventive efficacy of DHEA analog 8354 in these target tissues has been suggested by epidemiological data. As mentioned above, levels of DHEA (primarily as the sulfate) decrease steadily after 30 years of age in both men and women; conversely, both breast and prostate cancer increase with age [reviewed in 1]. Also, subnormal levels of DHEA and DHEA-sulfate are observed in women with breast cancer [*e.g.*, 14]. In a prospective study, 24-hour urinary steroid levels were obtained from 5,000 women on the island of Guernsey [15]. It was found that the excretion of DHEA metabolites was lower in women who developed breast tumors over the following nine years. In addition, urinary DHEA levels were significantly lower in women who developed ovarian cancer over 130 months [16].

PHARMACODYNAMICS

In rats, the lowest effective dose of DHEA analog 8354 in the rat mammary gland carcinogenesis model was 1 g/kg diet (*ca.* 0.2 mmol/kg-bw/day); the same dose of DHEA was effective against rat prostate carcinogenesis. In the same species, the 90-day NOEL was 250 mg/kg-bw/day (0.8 mmol/kg-bw/day). This 4-fold difference suggests that an effective dose without toxicity may be possible in breast and prostate cancer chemoprevention, although the therapeutic ratio is not as high as desired. In the rat colon cancer model, the lowest effective dose was an order of magnitude lower (100 ppm, or *ca.* 0.02 mmol/kg-bw/day), giving a more comfortable therapeutic ratio of 40.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

For clinical development of DHEA analog 8354, a reliable, sensitive and specific drug effect measurement should be identified. An obvious choice for evaluation is measurement of G6PDH activity, possibly in lymphocytes. Lymphocytes from carriers of hereditary G6PDH deficiency have an impaired ability to metabolize B(*a*)P.

Safety Issues

The major toxicological issues in humans are the potential for liver and other hormone-related effects. As with tamoxifen citrate, estrogenic effects such as uterine proliferation are a concern. No androgenic effects were found in preclinical studies.

Pharmacodynamics Issues

Determination of the safety margin for DHEA analog 8354 is a goal of Phase I and II clinical trials. In rats, the therapeutic ratio varied from 4–40, with the smallest ratio in the mammary glands for DHEA analog 8354 and in the prostate for DHEA.

Regulatory Issues

The CB-contracted 90-day subchronic toxicity studies of DHEA analog 8354 in rats and dogs may be sufficient to support Phase I clinical trials to investigate pharmacokinetics and safety. No chronic toxicity or carcinogenicity studies have been completed by the CB, except for specialized 6-month studies in gonadectomized animals to evaluate androgenic and estrogenic effects. In order to support longer Phase II trials, preclinical toxicity studies of comparable length must be performed in two species.

Supply and Formulation Issues

The viscosity of a suspension of the analog in oil appeared to alter absorption in preclinical pharmacokinetics studies. An alternate formulation of bulk drug will need to be developed for clinical trials. The rights to DHEA analog 8354 are held by Research Corporation Technology. Negotiations are in progress for an agreement to supply the agent.

Intermediate Biomarker Issues

DHEA analog 8354 has been shown to inhibit proliferation (measured as [^{3}H]-thymidine incorporation), histological lesions (papillomas) and car-

cinogen-DNA adducts in the two-stage mouse skin model of tumorigenesis. For clinical development of this agent, evaluation of its effect on premalignant lesions (actinic keratosis, dysplastic nevi) in human skin may be informative endpoints. In the Phase II trials under consideration for 1995, efficacy endpoints include modulation of intermediate biomarkers in breast and prostate cohorts.

Clinical Studies Issues

Three short-term Phase II chemoprevention trials in hormone-responsive tissues are under consideration by the CB for 1995, pending an agreement for drug supply and conduct of a Phase I trial. Two trials involve treatment of cancer patients in the period between diagnostic biopsy and surgery—the individual trials address breast and prostate cancer. The prostate trial will also accrue observation-only incidental cancer patients and PIN patients. A second prostate trial will evaluate DHEA analog 8354 in men at high risk for prostate cancer. A major aspect of these trials is dose-titration against intermediate biomarkers as potential surrogate endpoints for cancer incidence reduction.

REFERENCES

1. Gordon, G.B., Shantz, L.M., and Talalay, P. Modulation of growth, differentiation and carcinogenesis by dehydroepiandrosterone. *Adv. Enzyme Regul.* 26: 355–382, 1987.
2. Schwartz, A.G., Lewbart, M.L., and Pashko, L.L. Inhibition of tumorigenesis by dehydroepiandrosterone and structural analogs. In: Wattenberg, L., Lipkin, M., Boone, C.W., and Kelloff, G.J. (eds.), *Cancer Chemoprevention*, Boca Raton, FL: CRC Press, pp. 443–455, 1992.
3. Knudsen, J.F. and Mahesh, V.B. Initiation of precocious sexual maturation in the immature rat treated with dehydroepiandrosterone. *Endocrinology* 97: 458–468, 1975.
4. Yamada, J., Sakuma, M., Ikeda, T., Fukuda, K., and Suga, T. Characteristics of dehydroepiandrosterone as a peroxisome proliferator. *Biochim. Biophys. Acta* 1092: 233–243, 1991.
5. Schwartz, A.G., Lewbart, M.L., and Pashko, L.L. Novel dehydroepiandrosterone analogues with enhanced biological activity and reduced side effects in mice and rats. *Cancer Res.* 48: 4817–4822, 1988.
6. Pashko, L.L., Lewbart, M.L., and Schwartz, A.G. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-promoted skin tumor formation by 16α-fluoro-5-androsten-17-one and its reversal by deoxyribonucleosides. *Carcinogenesis* 12: 2189–2192, 1991.
7. Hastings, L.A., Pashko, L.L., Lewbart, M.L., and Schwartz, A.G. Dehydroepiandrosterone and two structural analogs inhibit 12-*O*-tetradecanoylphorbol-13-acetate stimulation of prostaglandin E_2 content in mouse skin. *Carcinogenesis* 9: 1099–1102, 1988.
8. Henderson, E., Yang, J.Y., and Schwartz, A. Dehydroepiandrosterone (DHEA) and synthetic DHEA analogs are modest inhibitors of HIV-1 IIIB replication. *AIDS Res. Hum. Retroviruses* 8: 625–631, 1992.
9. Ratko, T.A., Detrisac, C.J., Mehta, R.G., Kelloff, G.J., and Moon, R.C. Inhibition of rat mammary gland chemical carcinogenesis by dietary dehydroepiandrosterone or a fluorinated analogue of dehydroepiandrosterone. *Cancer Res.* 51: 481–486, 1991.
10. Schwartz, A.G., Fairman, D.K., Polansky, M., Lewbart, M.L., and Pashko, L.L. Inhibition of 7,12-dimethylbenz[*a*]anthracene-initiated and 12-*O*-tetradecanoylphorbol-13-acetate-promoted skin papilloma formation in mice by dehydroepiandrosterone and two synthetic analogs. *Carcinogenesis* 10: 1809–1813, 1989.
11. Rao, C.V., Tokumo, K., Rigotty, J., Zang, E., Kelloff, G., and Reddy, B.S. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, α-difluoromethylornithine, 16α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. *Cancer Res.* 51: 4528–4534, 1991.
12. Schwartz, A.G. and Pashko, L.L. Food restriction inhibits [^{3}H]-7,12-dimethylbenz(*a*)anthracene binding to mouse skin DNA and 12-*O*-tetradecanoylphorbol-13-acetate stimulation of epidermal [^{3}H]-thymidine incorporation. *Anticancer Res.* 6: 1279–1282, 1986.
13. Kelloff, G.J., Boone, C.W., Crowell, J.A., Steele, V.E., Lubet, R., and Sigman, C.C. Chemopreventive drug development: Perspectives and progress. *Cancer Epidemiol. Biomarkers Prev.* 3: 85–98, 1994.
14. Thomas, B.S., Kirby, P., Symes, E.K., and Wang, D.Y. Plasma dehydroepiandrosterone concentration in normal women and in patients with benign and malignant breast disease. *Eur. J. Cancer* 12: 405–409, 1976.
15. Bulbrook, R.D., Hayward, J.L., and Spicer, C.C. Relation between urinary androgen and corticoid excretion and subsequent breast cancer. *Lancet* 2: 395–398, 1971.
16. Cuzick, J., Bulstrode, J.C., Stratton, I., Thomas, B.S., Bulbrook, R.D., and Hayward, J.L. A prospective study of urinary androgen levels and ovarian cancer. *Int. J. Cancer* 32: 723–726, 1983.

Table I. Clinical Trials of DHEA Analog 8354 Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Doses Study Duration	Endpoints	Remarks
Phase II (Dose titration, efficacy, intermediate biomarkers)					
Planned Study Phase II DHEA Analog in Breast Neoplasia: Administration During the Period between Diagnostic Core Biopsy and Definitive Surgery 1995	Breast	Biopsy-proven breast cancer patients scheduled for surgery (dose and biomarker evaluation)	Oral; 1 week to 2 months	Dose-titration; intermediate biomarkers	Study not yet designed.
Planned Study Phase II 1995	Prostate	Patients at high risk for prostate cancer	Oral 6 months	Dose-titration; intermediate biomarkers	Study not yet designed.
Planned Study Phase II 1995	Prostate	Prostate cancer patients scheduled for prostatectomy; low grade/stage observation-only patients; PIN	Oral 2 years	Efficacy: Carcinoma, PIN, and other intermediate biomarkers	Study not yet designed.

DHEA ANALOG DEVELOPMENT STATUS