RESEARCH COMMUNICATION

Promotion of Liver Lesion Development in the Syrian Hamster by Dietary fat Following Multi-organ Initiation is Inhibited by DHEA-S Administration

Cheol Beom Park[1,2], Dae Joong Kim[1,3], Malcolm A Moore[1,4], Nobuo Takasuka[1], Hiroyuki Tsuda[1]

Abstract

The influence of dietary supplementation with dehydroepiandrosterone-sulphate (DHEA-S) at 0.6% was investigated in male Syrian golden hamsters initiated by treatments with azoxymethane(AOM), and dihydroxy-di-n-propyl nitrosamine (DHPN), timed after transfer from a choline-deficient to a normal diet. These carcinogens respectively target the colon, and the respiratory tract, the liver and pancreas. A total of 75 animals were divided into 6 groups, 1-3 (20 animals each) receiving the initiation protocol of during the first 8 weeks, and 4-6 (5 animals each) given the vehicles alone. The hamsters in groups 2,3 and 5,6 then received a high fat diet (20% corn oil) while DHEA-S was given in addition for 20 weeks to groups 3 and 6. Groups 1 and 4 served as controls on the basal diet. Assessment of development of preneoplastic and neoplastic lesions, after sacrifice at week 28, revealed increase in hepatocellular liver nodules and the index of BrdU incorporation in the hepatocellular cells of the liver in the initiated animals given the high fat diet. This was significantly reduced by the DHEA-S supplementation. Non-significant tendencies for high fat enhancement and hormone protection were also observed for lung and colon tumors.

Key words: hamster hepatocarcinogenesis - fat promotion - DHEA chemoprevention - wide spectrum inititiation

Asian Pacific J Cancer Prev, **1**, 329-332

Introduction

There is now a great deal of interest in chemoprevention of cancer development. One agent which has received attention in this respect is dehydroepiandrosterone, the adrenal steroid. Dietary administration has been found to exert inhibitory potential in a large number of animal models (Gordon et al., 1987; Schwartz et al., 199) and there are also data pointing to protective effects in humans (Gordon et al., 1987). However, it may also cause adverse effects, including liver neoplasms when given long-term to the rat (Rao et al., 1992). In assessing influence of potential chemopreventors it is clearly important that account be taken of organ or tissue-dependence. For this purpose, models using combinations of carcinogens targeting different organs can be employed.

In the present study, Syrian hamsters were initiated with DHPN and AOM to induce lesions in the hepatobiliary axis and the pancreas, as well as the lung and colorectum. A modification of the rapid induction model of Konis\hi and co-workers (Mizumoto et al., 1989; 1990) was selected as the inducing regimen.

Since fat consumption is reported to be associated with adenocarcinoma development in many organs (World Cancer Research Fund, 1997) , a 20% corn oil diet was administered to appropriate groups. This was followed by DHEA-Sulphate at 0.6% in the diet as a test agent..

Materials and Methods

Male Syrian hamsters were purchased at six weeks of age and maintained under constant conditions. After one week for acclimation, the experimental protocol was performed (see Figure 1). The carcinogens were commercially purchased and the DHEA-S was kindly supplied by Kanebo, Osaka. A number of animals died during the experiment,

1.Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan, Phone: +81-3-3542-2511 (Ext.4350), Fax: +81-3-3542-3586, e-mail:htsuda@gan2.ncc.go.jp 2. Department of Anatomy, Catholic University of Seoul, APOCP Training Centre, Phyathai 69/30, Ratchathewi, Bangkok 10400, Thailand

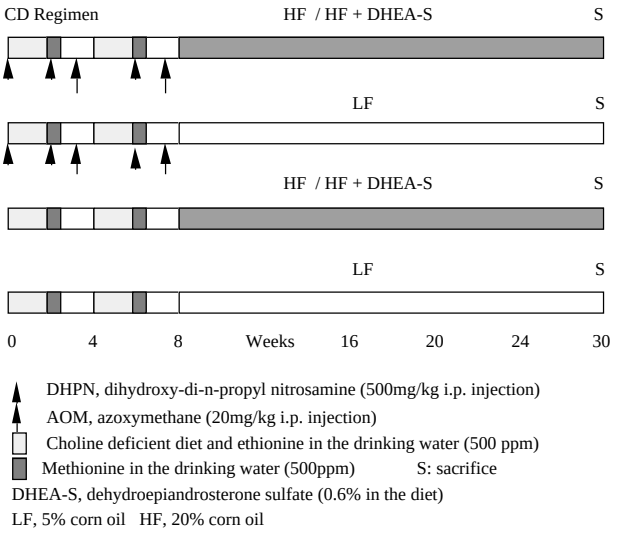


Figure 1. Experimental protocol

the survivors being sacrificed at the end of week 30. One hour prior to scarfice the hamsters received an i.p. injection of bromodeoxyuridine. Blood samples were taken from the abdominal aorta to allow assessment of insulin levels by radioimmunoassay. The major organs were removed and, after weighing in the liver case, fixed in formalin for routine processing and embedding in paraffin. For the intestines, they were opened for preparation of Swiss rolls.

The numbers of lesions were counted under a light microscope: these were nodules of as cells compressing the surrounding parenchya in the liver, atypical hyperplasias and nodules of the pancreas and lung and polyps in the colon and rectum. Immunohistochemically demonstrated BrdU staining allowed counts of incorporating hepatocytes in non-nodular liver areas.

Table 1. Body, Organ and Serum Insulin Data

Group	Number of animals	Body weight (g)	Relative liver weight (%)	Insulin
Control	3	165.0+13.4	4.1+0.3	2.96+0.39
High Fat	3	197.2+12.8	4.1+0.2	54.9+32.8
DHEA-S	4	223.2+28.6	3.8+0.4	35.5+17.9
Carc	13	164.0+25.8	4.3+0.5	4.43+4.63
C+HF	13	183.3+14.2	3.8+0.6	2.95+2.54
C+DHEA-S	17	171.5+18.7	4.0+0.3	14.8+14.3

Table: Organs for Which Modification of Tumorigenesis by DHEA has been Reported

Skin:	Rat, Pashko et al.,1984; 1985;
Lung:	Mouse, Schwartz and Tannen, 1981
Liver:	Rat, Garcea et al, 1988, Moore et al., 1988a,b
Gastric	Man, Gordon et al., 1993
Colon:	Rat, Hamilton et al., 1991; Rao et al., 1991; Mouse, Nyce et al., 1984
Breast:	Man, Barrett-Connor et al, 1990, Gordon et al., 1990, Helzlsouer et al., 1992; Rat, (radiation) Inano et al., 1995, (in liver) Li et al., 1994; Ratko et al., 1991; Mouse, Schwartz, 1979
Ovary:	Man, Heinonen et al., 1987
Uterine Cervix:	Mouse, Rao, 1989
Urinary Bladder:	Man, Gordon et al., 1981; Rat, Shibata et al., 1993;
Prostate:	Rat, Rao et al., 1999
Testis:	Rat, Rao et al., 1992
Thyroid: Rat,	Moore et al., 1986

Results

The results for body and liver weights, as well as insulin, are given in Table 1 and for incidence data in Table 2.There were no significant differences regarding weights. The insulin level appeared to be increased by high fat in the controls without carcinogen exposure but not in animals bearing lesions.

Development of liver nodules was significantly increased by the high fat diet, this being inhibited by DHEA-S. Similar non-significant tendencies were observed for the lung and colon, but not the pancreas. Percentages of hepatocytes incorporating BrdU were 2.1+1.3, 6.3+1.7 and 3.1+1.3 for the C+Basal diet, HF and HF + DHEA-S groups, the increase with HF and the reduction by the hormone both being significant ($p<0.05$).

Discussion

The present study provided evidence that DHEA-S can inhibit the development of liver nodules after DHPN and AOM initiation, associated with a decrease in the cell turnover. Links to proliferation and its suppression have been reported earlier (Pashko et al., 1981; Garcea et al., 1988;

Table 2. Incidence and Multiplicity Data

Group	Number of animals	Liver Nodules %Inc	Liver Nodules No/Hamster	Lung Nodules %Inc	Lung Nodules No/Hamster	Pancreatic Lesions %Inc	Pancreatic Lesions No/Hamster	Colon Polyps %Inc	Colon Polyps No/Hamster
Carc	13	7.7	0.08*	54	0.62*	15	0.15	69	0.9
C + HF	13	39	0.61	77	1.15	38	0.38	85	1.6
C + DHEA-S	17	29	0.29*	71	0.94	35	0.59	69	1.1

Significantly different from the C+HF value at $p<0.05$

Shulz and Nyce, 1994).

DHEA and now its sulphate, DHEA-S, have been shown to exert modifying effects on tumorigenesis in very many organs (see the Table). Other hypolipidemic agents have also been shown to have protective actions (Maruyama et al., 1994; Mizumoto et al., 1988).

Clearly the adrenal steroid DHEA itself is not an agent that promises universal benefit for all ailments (Pugh et al, 1999). However, the interplay between this hormone and others in the body, including insulin, has yet to be elucidated in detail, although a great deal is known, for example, about effects in the liver (Belli, et al., 1992). In the present study the data for serum insulin were very difficult to interpret, since there was no consistency. The fact that the expected rise in insulin could be confirmed in a parallel experiment looking at starvation-refeeding (data not shown) might suggest that the high fat diet did induce an increase, but that this was masked by other influences in animals bearing neoplastic lesions.

The present findings offer some support for beneficial actions of DHEA, generally in line with the literature. The question now is whether more interest should be placed in this and similar compounds acting in concert with other chemopreventors. Suitable markers for assessment of different possibilities are now required.

Acknowledgements

The authors would like to express their appreciation to Dr Norio Awata of Kanebo Ltd, Japan, for the kind gift of DHEA-S and DHEA for the present work. During the performance of this study , Cheol Beom Park and Malcolm Moore were recipients of Research Fellowships from the Foundation for Promotion of Cancer Research Program for Invitation of Foreign Researchers, Japan. This work was supported in part by a Grant-in-Aid for the Second Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare, by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare,

References

Barrett-Connor E, Frisdlander NJ, Khaw K-T (1990). Dehydroepiandrosterone sulfate and breast cancer risk. *Cancer Res*, **50**, 6571-4.

Belli M, Battelli D, Fornieri C, et al (1992). Changes in liver structure and function after short term and long term treatment of rats with dehydroepiandrosterone. *J Nutr*, **122**, 967-76.

Birt DF, Pour PM (1985). Effects of interaction of dietary fat and protein on N-nitrosobis(2-oxopropyl)amine-induced carcinogenesis and spontaneous lesions in Syrian golden hamsters. *J Natl Cancer Inst*, **74**, 1121-1127.

Garcea R, Daino L, Pascale R, et al (1988) Reversal by ribo- and deoxyribonucleosides of dehydroepiandrosterone-induced inhibition of enzyme altered foci in the liver of rats subjected to the initiation-selection process of experimental carcinogenesis. *Carcinogenesis*, **9**, 931-8.

Gordon GB, Bush TL, Helzlsouer KJ, Miller SR, Comstock GW (1990). Relationship of serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate to the risk of developing postmenopausal breast cancer. *Cancer Res*, **50**, 3859-62.

Gordon GB, Helzlsouer KJ, Alberg AJ, Comstock GW (1993). Serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate and the risk of developing gastric cancer. *Cancer Epidemiol Biomarkers Prev*, **2**, 33-5.

Gordon GB, Helzlsouer KJ, Comstock GW (1991). Serum levels of dehydroepiandrosterone and its sulfate and the risk of developing bladder cancer. *Cancer Res*, **51**, 1366-9.

Gordon GB, Shantz LM, Talalay P (1987) Modulation of growth, differentiation, and carcinogenesis by dehydroepiandrosterone. *Adv Enzyme Reg*, **26**, 355-82.

Hamilton SR, Gordon GB, Floyd J, Golightly S (1991). Evaluation of dehydroepiandrosterone for chemoprevention against tumorigenesis in premalignant colonic epithelium of male F344 rats. *Cancer Res*, **51**, 476-80.

Heinonen PK, Koivula T, Pystynen P (1987). Decreased serum level of dehydroepiandrosterone sulfate in postmenopausal women with ovarian cancer. *Gynecol Obstet Invest*, **23**, 271-4.

Helzlsouer KJ, Gordon GB, Alberg AJ, Bush TL, Comstock GW (1992). Relationship of prediagnostic serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate to the risk of developing promenopausal breast cancer. *Cancer Res*, **52**, 1-4.

Inano H, Ishii-Ohba H, Suzuki K (1995). Chemoprevention by dietary dehydroepiandrosterone against promotion/progression phase of radiation-induced mammary tumorigenesis in rats. *J Steroid Biochem Mol Biol*, **54**, 47-53

Kazakoff K, Cardesa T, Liu, J et al (1996). Effects of voluntary physical exercise on high-fat diet-promoted pancreatic carcinogenesis in the hamster model. *Nutr Cancer*, **26**, 265-79.

Kloeppel, G., Bommer, G., Rueckert, K. and Seifert, G. (1980) Intraductal proliferation in the pancreas and its relationship to human and experimental carcinogenesis. *Virchow Archiv. A*, **387**, 221-233.

Li S, Yan X, Belanger A, Labrie F (1994). Prevention by dehydroepiandrosterone of the development of mammary carcinoma induced by 7,12-dimethyl(a)benzanthracene in adult rat liver. *Breast Cancer Res Treat*, **29**, 203-217.

Maruyama H, Amanuma T, Tsutsumi M, et al (1994). Inhibition by catechol and di(2-ethylhexyl)phthalate of pancreatic carcinogenesis after initiation with N-nitrosobis(2-hydroxypropyl)amine in Syrian hamsters. *Carcinogenesis*, **15**, 1193-6.

McCormick DL, Rao KV (1999). Chemoprevention of hormone-dependent prostate cancer in the Wistar-Unilever rat. *Eur Urol*, **35**, 464-7.

Mizumoto K, Tsutsumi M, Kitazawa S, Denda A, Konishi Y (1990). Usefulness of rapid production model for pancreatic carcinoma in male hamsters. *Cancer Lett*, **49**, 211-5.

Mizumoto K, Kitazawa S, Ito S, et al (1989). Cycles of repeated augmentation pressure in rapid production of pancreatic and cholangiocellular carcinomas in hamsters initiated with N-nitrosobis(2-oxopropyl) amine. *Carcinogenesis*, **10**, 1457-9.

Mizumoto, K., Kitazawa, S., Eguchi, T, et al (1988). Modulation of N-nitrosobis (2-hydroxypropyl) amine-induced carcinogenesis by clofibrate in hamsters. *Carcinogenesis*, **9**, 1417-20.

Moore MA, Thamavit W, Tsuda, H, et al (1986). Modifying influence of dehydroepiandrosterone on the development of di-hydroxy-di-n-propylnitrosamine initiated lesions in the thyroid and liver of F344 rats. *Carcinogenesis*, **7**, 311-6.

Moore MA, Weber E, Bannasch P (1988). Modulating influence of dehydroepiandrosterone administration on the morphology and enzyme phenotype of dimethylaminoazobenzene-induced hepatocellular foci and nodules. *Virch Arch B Cell Pathol*, **55**, 337-43.

Moore MA, Weber E, Thornton M, Bannasch P (1988). Sex dependent tissue-specific opposing effects of dehydroepiandrosterone on initiation and modulation stages of liver and lung carcinogenesis induced by dihydroxy-di-n-propylnitrosamine in F344 rats. *Carcinogenesis*, **9**, 1507-9.

Nyce JW, Magee PN, Hard GC, Schwartz AG (1984). Inhibition of 1,2-dimethylhydrazine-induced colon carcinogenesis in Balb/C mice by dehydroepiandrosterone. *Carcinogenesis*, **5**, 57-62.

Pascale RM, Simile MM, De Miglio MR, et al (1995). Inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase activity and gene expression by dehydroepiandrosterone in preneoplastic liver nodules. *Carcinogenesis*, **16**, 1537-42.

Pashko LL, Hard GC, Rovito RJ, et al (1985). Inhibition of 7,12-dimethylbenz[a]anthracene-induced skin papillomas and carcinomas by dehydroepiandrosterone and 3-methylandrost-5-en-17-one in mice. *Cancer Res*, **45**, 164-6.

Pashko LL, Rovito RJ, Williams JR, Sobel EL, Schwartz AG (1984). Dehydroepiandrosterone (DHEA) and 3-methylandrost-5-en-17-one: inhibitors of 7,12-dimethylbenz[a]anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol-13-acetate (TPA)-promoted skin papilloma formation in mice. *Carcinogenesis*, **5**, 463-6.

Pashko LL, Schwartz AG, Abou-Garbia M, Swern D (1981). Inhibition of DNA synthesis in mouse epidermis and breast epithelium by epiandrosterone and related steroids. *Carcinogenesis*, **2**, 717-21.

Pour PM, Krueger FW, Cardesa A, Althoff J, Mohr U (1975). The effect of beta-oxidized-nitrosamines on Syrian golden hamsters. III. 2,2'-Dihydroxy-di-n-propylnitrosamine. *J Natl Cancer Inst*, **54**, 141-6.

Pugh TD, Oberley TD, Weindruch R (1999). Dietary intervention at middle age: caloric restriction but not dehydroepiandrosterone sulfate increases lifespan and lifetime cancer incidence in mice. *Cancer Res*, **59**, 1642-8.

Rao AR (1989). Inhibitory action of dehydroepiandrosterone on methylcholanthrene-induced carcinogenesis in the uterine cervix of the mouse. *Cancer Letters*, **45**, 1-5.

Rao CV, Tokumo K, Rigotty J, et al (1991). Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, a-difluoromethylornithine, 16a-fluoro-5-androsteron-17-one, and ellagic acid individually and in combination. *Cancer Res*, **51**, 4528-34.

Rao KV, Johnson WD, Bosland MC, et al (1999). Chemoprevention of rat prostate carcinogenesis by early and delayed administration of dehydroepiandrosterone. *Cancer Res*, 59, 3084-9.

Rao MS, Subbarao V, Yeldandi AV, Reddy JK (1992). Inhibition of spontaneous testicular Leydig cell tumor development in F-344 rats bydehydroepiandrosterone. *Cancer Lett*, **65**, 123-6.

Rao MS, Subbarao V, Yeldani AV, Reddy JK (1992). Hepatocarcinogenicity of dehydroepiandrosterone in the rat. *Cancer Res*, **52**, 2977-9.

Ratko TA, Detrisac CJ, Mehta RG, Kellof GJ, Moon RC (1991). Inhibition of rat mammary gland chemical carcinogenesis by dietary dehydroepiandrosterone or a fluorinated analogue of dehydroepiandrosterone. *Cancer Res*, **51**, 481-6.

Schulz S, Nyce J.W. (1994). Inhibition of protein farnesyl-transferase: a possible mechanism of tumor prevention by dehydroepiandrosterone sulfate. *Carcinogenesis*, **15**, 2649-52.

Schwartz AG (1979). Inhibition of spontaneous breast cancer formation in female C3H-Avy/A mice by long term treatment with dehydroepiandrosterone. *Cancer Res*, **39**, 1129-32.

Schwartz AG, Tannen RH (1981). Inhibition of 7,12-dimethylbenz-[a]anthracene- and urethan-induced lung tumor formation in A/J mice by long term treatment with dehydroepiandrosterone. *Carcinogenesis*, **2**, 1335-7.

Schwartz AG, Whitcomb JM, Nyce JW, Lewbart ML, Pashko LL (1988). Dehydroepiandrosterone and structural analogs: a new class of chemopreventive agents. *Adv Cancer Res*, **51**, 391-424.

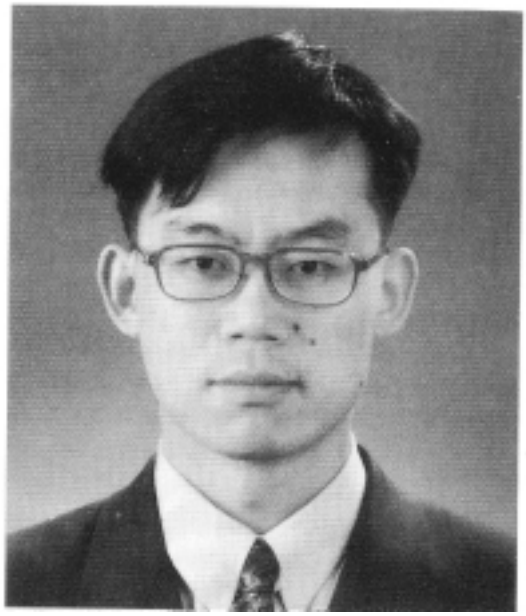
Shibata MA, Shirai T, Asakawa E, Hirose M, Fukushima S (1993). Inhibition by dehydroepiandrosterone of butylated hydroxyanisole (BHA) promotion of rat-bladder carcinogenesis and enhancement of BHA-induced forestomach hyperplasia. *Int J Cancer*, **53**, 819-23.

Svec F, Porter JR (1998). The actions of exogenous dehydroepiandrosterone in experimental animals and humans. *Proc Soc Exp Biol Med*, **218**, 174-91.

World Cancer Research Fund, American Institute for Cancer Research. Food, Nutrition and the Prevention of Cancer 1997.

Personal Profile: Cheom Beol Park

Dr Park obtained his Ph.D in the Veterinary Medicine Faculty, Seoul National University in 1999.



His major work experience has been in the Korean National Institute for Toxicological Research, as well as the Korean Environmental Management Research Office. For a two year period in the late 1990s he visited the Japanese National Cancer Center, conducting research in chemoprevention of cancer under Dr Hiroyuki Tsuda in the Experimental Pathology and Chemotherapy Division

Dr Park presently has a position at the Catholic Medical University in Seoul, where he is engaged in toxicological research related to NOS.