Serum Levels of Dehydroepiandrosterone and Its Sulfate and the Risk of Developing Bladder Cancer

Gary B. Gordon, Kathy J. Helzlsouer, and George W. Comstock

ABSTRACT

Dehydroepiandrosterone and dehydroepiandrosterone sulfate are endogenous steroids largely produced in the adrenal gland and excreted in the urine. Many studies have demonstrated that administration of dehydroepiandrosterone to animals protects against a variety of chemical carcinogens. Epidemiological studies suggest that the circulating levels of these steroids in humans are related to the risk of developing some cancers and of dying from atherosclerotic cardiovascular disease. We measured serum levels of both of these steroids in 35 individuals who donated serum to a community-based serum bank in 1974 and who subsequently developed bladder cancer and in 69 matched controls from the same cohort of volunteers. Prediagnostic serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate were significantly lower among cases compared with controls. The risk of developing bladder cancer increased monotonically with decreasing serum levels of both steroids. The observed associations were not affected by adjustment for smoking or the time interval between serum collection and diagnosis. These results support a role for dehydroepiandrosterone and/or dehydroepiandrosterone sulfate in the prevention of bladder cancer.

INTRODUCTION

Bladder cancer, the sixth most common cancer in the United States, will account for over 10,000 deaths in 1990 (1). Although the etiology of bladder cancer is not certain, a number of modifiable host and environmental risk factors have been identified. These include cigarette smoking, radiation, schistosomiasis, and exposure to specific chemical carcinogens such as 2-naphthylamine (2, 3). Other suspected bladder carcinogens include saccharin and coffee (4-7). Exogenous factors that may reduce the incidence of bladder cancer include a high dietary intake of retinol, β-carotene, and other retinoids (8-20). In addition, high levels of selenium in serum appear to be associated with a decreased risk of developing bladder cancer (21). In an experimental rat model, the addition of DHEA to the diet reduces the incidence of papillary and nodular hyperplasia in the urinary bladder resulting from exposure to dihydroxy-di-n-propylinitrosamine (22). Therefore we investigated the possibility that serum levels of the endogenous anticarcinogenic steroid, DHEA, and its sulfate, DHEAS, might be associated with the risk of developing bladder cancer.

DHEA and DHEAS are endogenous steroids produced in the adrenal gland in response to adrenocorticotropic hormone. Substantial amounts of these steroids, particularly DHEAS, are excreted in the urine (for review, see Ref. 23). With the exception of cholesterol, DHEA is the most abundant steroid in the circulation, and its levels are 300 to 500 times higher than those of DHEA. Circulating levels of these steroids reach a peak in individuals of both sexes at about age 20 to 25 and thereafter decline continuously with age (24, 25). The profound decline with age has led to investigation of the intriguing possibility that serum levels of DHEA and DHEAS are related to the development of age-associated diseases such as cancer and atherosclerosis. This hypothesis is supported, in general, by epidemiologic studies showing an association of low serum or urinary levels of DHEA and/or DHEAS with the presence or risk of developing cancer (26-29) and with increased mortality from cardiovascular disease (30). However, women with early operable postmenopausal breast cancer have higher 24-h mean plasma levels of these steroids than do healthy controls (28), and high serum levels are also associated with an increased risk of developing postmenopausal breast cancer (31). Nonetheless, animal experiments provide additional support for the protective effect of DHEA. In rodents, administration of DHEA reduces the incidence of spontaneous and carcinogen-induced tumors, retards the development of atherosclerosis, and increases life span (32-34). We hypothesized, therefore, that the bladder lining, where most bladder cancers arise, might be uniquely protected by DHEA by virtue of its exposure to DHEA and DHEAS from both the circulation and the urine.

The present study was designed to assess the association between circulating levels of DHEA and DHEAS and the risk of developing bladder cancer. We compared serum levels of DHEA and DHEAS in individuals who subsequently developed bladder cancer with serum levels from matched controls without bladder or other cancers. Serum samples from both cases and controls were obtained from the Washington County serum bank. This serum bank is located in Maryland and was established in 1974.

MATERIALS AND METHODS

A nested case-control study was conducted with use of serum samples from a cohort of Washington County residents who participated in the establishment of a serum bank in 1974. A private census with an estimated coverage of 90% of county residents was carried out in the summer of 1975 to ascertain the extent of participation in the blood collection drive (21).

Serum samples were collected from 25,802 participants. Of these samples, 20,305 (representing 30% of the adult county residents) were collected from persons enumerated in the private census and were included in the cohort used for this study. Women, nonsmokers, and better educated individuals were more likely to have donated samples. The age range of the participants was 11 to 98 yr. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. 1 The investigation was supported by Grants CA 36390 and PO1 CA 44530 from the National Cancer Institute, Department of Health and Human Services, and by a Special Institutional Grant from the American Cancer Society (SIG-3). G. B. G. is a recipient of a W. M. Keck Foundation Scientist Award from The Johns Hopkins University School of Medicine. K. J. H. is a recipient of Preventive Oncology Academic Award IK07CA01522-01 from the National Cancer Institute, Department of Health and Human Services. G. W. C. is a recipient of Research Career Award HL 21670 from the National Heart, Lung, and Blood Institute, NIH.

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3 The abbreviations used are: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; CI, confidence interval.

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Incident bladder cancer cases were identified through discharge records from Washington County Hospital, the county's only general hospital, and from death certificates of county residents. During the period from January 1975 to June 1986 inclusive, 44 new cases of bladder cancer were identified among the study population. Completeness of ascertainment was assessed by comparing the number of observed cases with the number expected at the 1978–1981 race-sex-age-specific rates for the Surveillance, Epidemiology and End Results Program (SEER) registries (exclusive of Puerto Rico) to the estimated midpoint population of the cohort. The observed:expected ratio was 1.04. Of the 44 cases, 9 had to be excluded because their sera had been used in previous studies or they had a previous cancer. For each study case, the pathology report was reviewed. Transitional cell carcinomas served cases with the number expected at the 1978-1981 race-sex-age-cases for measurement of DHEAS levels, and in 34 cases for DHEA.

Laboratory Assays. Serum was prepared from blood samples within a few hours of blood collection and always within 24 h. The sera were then maintained at −70°C until they were thawed for the preparation of aliquots for another study. The thawed samples were maintained on ice for less than 3 h before refreezing at −70°C and then were maintained at −70°C until DHEA and DHEAS analyses were performed. Coded sets consisting of a case and its matched controls were assayed on the same day. Quality control samples were included in some sets. All assays were done in triplicate, and the laboratory personnel were unaware of the coding within sets. If a sample required replication, the entire set was reassayed.

DHEA and DHEAS assays were performed with radioimmunoassay kits from Wien Laboratories, Succasunna, NJ. These reagents were selected by comparison with combined chromatographic and enzymatic assays for DHEA and DHEAS (35). The kits were used as suggested by the manufacturer except that DHEA was extracted from serum with a 1:1 (v/v) mixture of dichloromethane and hexane. Distilled water extraction blanks were included in each DHEA assay. Known quality control samples were included in each assay. Standards were assayed in quadruplicate, and maximum binding was determined in six samples per assay. A logit regression was used to calculate the concentration of steroid in the samples. Inter- and intraassay variation was less than 10% for both DHEA and DHEAS.

Statistical Analysis. The SAS Statistical Package was used for analyses. DHEA and DHEAS levels were treated as both continuous and categorical exposure variables. The PROC analysis of variance procedure, which performs a paired t test allowing for 2:1 matching, was used to assess the mean difference in serum hormone levels between cases and controls. Because the distributions for DHEA and DHEAS are skewed, natural log transformations were used for statistical tests examining DHEA and DHEAS as continuous exposure variables. Conditional logistic regression analysis was used to assess and adjust for potential confounders of the association between DHEA and DHEAS levels and the risk of bladder cancer.

Tertile cutpoints for DHEA and DHEAS were based on the distribution of the hormone levels in the control population. Odds ratios were calculated from the regression coefficients, with the highest category of hormone levels as the reference value. Monotonic trends for the risk estimates were evaluated by the regression coefficients when the hormones were examined in the model as continuous variables. A 95% CI was used for significance testing. All P values are based on two-tailed tests.

RESULTS

Comparisons of cases and controls on matching criteria and other potentially confounding characteristics are shown in Table 1. As a result of matching, cases and controls had the same sex-race composition (all cases were white), age, and time interval between blood collection and the previous meal. None of the observed differences, including the smoking history, was statistically significant at the 95% level of confidence.

Table 2 shows the mean levels and the range of values of DHEA and DHEAS among cases and controls. Cases tend to have lower levels for both DHEA and DHEAS, but this is more pronounced for DHEA.

Because smoking has been reported to be associated with both DHEA and DHEAS levels and the risk of bladder cancer (2, 3), it is a potential confounding variable. Among the controls, current smokers at the time of blood collection tended to have higher levels of DHEAS, but the correlation between DHEA levels and the number of cigarettes smoked per day was not statistically significant (r = 0.16, P = 0.3). DHEA levels were not correlated with the number of cigarettes smoked per day (r = 0.001, P = 0.99). Adjustment for the effects of cigarette smoking at the time of blood collection was made by using the categories of ever versus never smokers as well as by the categories of current smokers in 1974, former smokers, and never smokers. Because none of these adjustments altered the observed associations between DHEA and DHEAS levels and the risk of developing bladder cancer, only the unadjusted odds ratios are presented in Fig. 1. The risk of bladder cancer increased with decreasing levels of both DHEA and DHEAS. The trends for DHEA and DHEAS increased monotonically (P = 0.06 and P = 0.04, respectively). Similar analyses were done treating DHEA and DHEAS levels as continuous variables. The estimated risk of developing bladder cancer increases 2-fold for every 1-log unit of decrease in DHEA (pmol/ml)
SERUM LEVELS OF DHEA(S) AND THE RISK OF BLADDER CANCER

Fig. 1. Odds ratios of developing bladder cancer according to serum DHEA and DHEAS levels. □, highest tertile; □, middle tertile; □, lowest tertile. The highest tertile is the reference category. Middle tertile values for DHEA are 6.31 to 10.18 pmol/ml inclusive; the odds ratio is 2.84 (95% confidence interval, 0.83 to 9.74); the lowest tertile odds ratio is 4.01 (95% confidence interval, 1.19 to 14.54); and test for trend P = 0.06. Middle tertile values for DHEAS are 2.50 to 4.23 nmol/ml inclusive; the odds ratio is 2.19 (95% confidence interval, 0.72 to 6.66); the highest tertile odds ratio is 3.74 (95% confidence interval, 1.07 to 13.08); and test for trend P = 0.04.

(95% CI, 0.97 to 4.54) and DHEAS (nmol/ml) (95% CI, 1.03 to 3.70).

Since cancer may have been present at the time of blood collection in some cases and some of the observed effects might be related to the presence of the cancer, we examined the results of stratifying the cases by early diagnosis (those diagnosed within 2 yr of blood collection, 5 cases) and late diagnosis (those diagnosed more than 2 yr from the time of blood collection, 30 cases). The observed protective effects of DHEA and DHEAS against bladder cancer were even stronger with the exclusion of cases diagnosed within 2 yr of donating serum. The odds ratios for the development of bladder cancer among cases diagnosed after 2 yr for the middle and low tertiles compared with the highest tertiles were as follows: for DHEA, 3.53 (95% CI, 0.87 to 14.36) and 4.87 (95% CI, 1.26 to 20.40), respectively; and for DHEAS, 2.21 (95% CI, 0.65 to 7.54) and 5.82 (95% CI, 1.36 to 24.04), respectively. Similar relationships between serum levels of DHEA and DHEAS and the risk of bladder cancer were observed for both men and women.

DISCUSSION

The results of this prospective study indicate that in both men and women high serum levels of DHEA and DHEAS are associated with a decreased risk of developing bladder cancer. We are unaware of any other study that has examined the association between DHEA and DHEAS and the development of bladder cancer in humans. DHEA and DHEAS can be converted to potent estrogens and androgens, and it is thought that such steroids might have a role in the development or progression of bladder cancer (37). For example, bladder cancer is found more commonly and may have a more aggressive behavior in men (1, 37, 38). There are also examples of an increased susceptibility of male animals to spontaneous and chemically induced bladder cancers (39, 40). In one such study, castration of males or testosterone supplementation of male castrates or normal females minimized the gender-related differences in tumor occurrence (40). However, there are other models in which female animals are more susceptible to bladder carcinogens (41). Thus, the evidence directly implicating androgenic and estrogentic steroids in the etiology or progression of bladder cancer remains incomplete. Although formal studies of the effect of DHEA as a chemoprotective agent in animal models of bladder cancer are currently under way (42), the incidence of bladder nodular and papillary hyperplasia in male rats exposed to dihydroxy-di-n-propylaminoxamine is reduced by the inclusion of DHEA in the diet (22). We believe, therefore, that these serological findings are consistent with the results of laboratory observations.

The mechanism by which DHEA/DHEAS exerts this protective action is not known, but the protective effect may be related to the ability of DHEA to inhibit cellular proliferation and differentiation and to alterations in the metabolism of various carcinogens with the resultant decreased binding to DNA (32-34). It has been suggested that these actions are secondary to the competitive inhibition of glucose-6-phosphate dehydrogenase by DHEA and the subsequent decreased availability of 5-carbon sugars for the synthesis of ribo- and deoxyribonucleosides and of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for xenobiotic metabolism and biosynthetic processes. In fact, such inhibition of glucose-6-phosphate dehydrogenase appears to be responsible for the block of differentiation of 3T3-L1 preadipocytes exposed to DHEA and related steroids (43).

The genitourinary tract and the lining of the bladder, in particular, may have a unique level of exposure to DHEA and DHEAS and, hence, this site might be particularly suitable as a target for chemoprotection with DHEA. Alternative explanations for our findings should also be considered. In any cohort study the possible effects of a bias introduced by different losses to follow-up among cases and controls should be considered. However, the Washington County population has a low rate of outmigration (1% per year), and case ascertainment is estimated to be reasonably complete. Therefore this is unlikely to affect the observed results of this study. Also, the observed association may not be a direct protective effect by DHEA or DHEAS but rather an effect of some other factor(s) for which it is a surrogate marker.

Despite the small size of the study and its limited power, the findings of a significant quantitative association between DHEA and DHEAS and the risk of bladder cancer, together with a dose-response relationship, are consistent with a chemoprotective effect of these adrenal steroids against bladder cancer. Nevertheless, as in all observational studies, it is important to determine whether these findings can be replicated in animal models and other human studies.

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