DETECTING AN ASSOCIATION BETWEEN PROSTATE CANCER OCCURRENCE AND TCDD EXPOSURE IN THE U.S. VIETNAM VETERAN POPULATION

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Introduction

In the 1996 and 1998 Updates on Veterans and Agent Orange, the Institute of Medicine's Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides cited "limited/suggestive evidence of an association between exposure to herbicides and prostate cancer[1,2]. The Secretary of the Department of Veterans Affairs (VA) added prostate cancer to the list of conditions recognized for presumption of service-connection for Vietnam veterans based on exposure to herbicides containing dioxin. Most herbicides used during the Vietnam Conflict contained the toxic contaminant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The evidence for an association between TCDD exposure and prostate cancer derives mostly from occupational cohorts other than Vietnam veterans, which show a small but significant increase in prostate cancer mortality[1,2,3]. Compared with the size of the population of known U.S. Vietnam veterans, (over 2.8 million males), the occupational and community cohorts studied thus far have been relatively small or of young average ages with few deaths expected from prostate cancer. For example, the Air Force's Ranch Hand Study reported a 4-fold increased risk in prostate cancer mortality associated with elevated serum TCDD levels, but the estimate was based on only 2 deaths[4]. On the other hand, a recent report of a large cohort of 33,658 pesticide applicators followed for mortality since 1975 found a significant 2.4-fold increased risk of prostate cancer[5]. Conclusive evidence associating herbicide exposure with prostate cancer may be easier to obtain from larger cohorts using the more common endpoint, prostate cancer incidence rather than mortality.

The prevalence and severity of exposure to TCDD-contaminated herbicides in the Vietnam veteran population remain unknown. TCDD assays first became available in the late 1980s and the costs have remained prohibitive for large-scale use. Estimates of elevated TCDD levels based on blood or adipose tissue collected 20 or more years after the War have been as low as 1-3% in small samples of Vietnam veterans[6]. In contrast, the VA's Agent Orange Registry includes about 10% of the entire Vietnam veteran population[1]. Surveys of Vietnam veterans who were not part of the Ranch Hand or Army Chemical Corps groups (with known high herbicide exposure) indicate that 25 to 55% of the population believes they were substantially exposed to herbicides. In a population-based study of birth defects in offspring, the CDC estimated a 15% prevalence of herbicide exposure among Vietnam veteran fathers[7]. With a population of 28,000 to 420,000 exposed and aging U.S. Vietnam veterans, increases in prostate cancer incidence should be detectable.

TCDD is a ubiquitous environmental contaminant that induces a broad spectrum of biochemical and toxic responses involving multiple endocrine pathways[3,8,9]. Genetic susceptibility may mediate the adverse health effects of TCDD. Thus, the relationship between human cancers and TCDD may not follow a linear dose response pattern. TCDD has been reported to cause rapid down-regulation of nuclear estrogen receptor levels and induction of cytochrome P450 enzymes (*i.e.*, CYP1A1, CYP1A2, and CYP1B1), which leads to the rapid oxidative metabolism of both 17ß-estradiol and testosterone[1,2,10,11]. Many believe that the initial and most important steps in the de-

velopment of adverse health effects from TCDD involve the aryl hydrocarbon receptor (*AhR)* gene and its gene product[12,13]. Kawajiri and colleagues recently identified a human *AhR* polymorphism (substitution of Arginine by Lysine)[14]. Although the allele frequency was 0.12 in a Caucasian population, it was 0.41 in the African American group tested. If TCDD-or other aryl hydrocarbon-induced CYP activity contributes to prostate carcinogenesis, reported high rates of prostate cancer in African American men and in black men from other countries may be due in part to racial differences in the allele frequencies of *AhR* and *CYP* polymorphisms.

The goal of this nation-wide population-based study is to determine the contribution of exposure to herbicides, genetic susceptibility to TCDD exposure, or TCDD-induced endocrine disruption through one or more metabolic pathways to the development or progression of prostate cancer in Vietnam veterans. Specific aims are to

1. Determine the association of prostate cancer incidence with quantitative estimates of exposure to herbicides (including serum TCDD and structural analogs), and the potential for genetic susceptibility at the following polymorphic loci: *CYP1A1, CYP1A2, CYP1B1,* and the TCDD gene-induction gatekeeper, the *AhR.*
2. Examine how serum levels of TCDD/structural analogs relate to prostate tissue expression of *ERα, ERβ,* (estrogen receptor) and *AR* (androgen receptor) based on mRNA transcript levels, and *CYP1A1* and *CYP1B1* based on immunohistochemistry.
3. Examine correlations of levels of CYP phenotypes with serum TCDD and its structural analogs both within and between groups of prostate cancer cases and controls.

Methods and Materials

The study is divided into two Phases. In Phase I, we propose to identify at least 2,916 possible cases of prostate cancer by linking a database of 2.8 million Vietnam veterans with Registries of the National Cancer Institute's (NCI) Surveillance, Epidemiology and End Results (SEER) Program and other States for the years 1996 through 2000. To estimate the minimum number of possible cases to be ascertained, we assumed an average annual prostate cancer incidence rate of 60 per 100,000 person-years. We further assumed that participant Registries would cover at least 40% of the total U.S. male Vietnam veteran population. This is a conservative estimate as our coverage in an EPA-funded study with a very similar design (enrolling Vietnam veterans who have parented a child with a neural tube defect) is actually 85%. We also assume that prostate cancer ascertainment will be at least 90% complete over the 5 year study period. Our database currently includes over 2.8 million Vietnam veterans. We assume that about 2.7 million male Vietnam veterans would remain at risk of prostate cancer between 1996 and 2000. Finally, we assume no increased incidence of prostate cancer in the population.

We will select potential controls from the Vietnam veteran database using a stratified random procedure. Records that "match" a Cancer Registry record will be excluded from the database of "potential controls." Random numbers will be generated and assigned to each record in the potential control pool. Potential controls will be frequency-matched to each case on birth year, race, home-State and military pay grade. We will select at least 2,916 potential controls. We will obtain addresses for all study candidates through the IRS and a commercial tracing agency. In recruiting study participants, we will send all study candidates the same letter inviting them to participate in the study if they served on active duty in the U.S. military in Southeast Asia between 1962 and 1975. We anticipate mailing at least 5,832 surveys. Based on our experience with our EPA-funded study, we expect a 60% enrollment rate. A mailed, self-administered survey will collect information on medical and military histories. We will use survey responses to assign veterans into 3 groups of possible herbicide exposure: high, moderate, or low. Exposure assessment models are currently under development (and will be validated with serum TCDD levels) as part of our EPA study. The exposure assessment modeling will enable us to use an economical "two-stage" sampling approach to examine the association of prostate cancer risk with molecular biomarkers in Phase II of the study.

In Phase II, we will enroll a subset of 225 cases and 225 age- and ethnicity-matched controls selected from the Phase I participants in a stratified-random manner to ensure equal numbers of veterans from each of the 3 estimated herbicide exposure opportunity groups. Cases and controls will be asked to submit blood samples for serum TCDD/structural analog levels and for genotyping of *CYP1A1, CYP1A2, CYP1B1* and the *AhR*.. The blood sample will also enable phenotyping of CYP1A1 and CYP1B1 by immunoblotting. Phase II participants will be asked to submit a urine sample (following ingestion of a standard caffeine dose) for CYP1A2 phenotyping. Blood and urine samples will be collected at the participant's location, date and time of choice. Medical records, tissue-paraffin blocks and histochemically-stained slides from diagnostic biopsies of the prostate or from surgical prostatectomies will be sought from health providers and source-pathology laboratories when authorized by the individual participants. Tissue paraffin blocks will be assayed for expression of estrogen and androgen receptor mRNA transcripts and of CYP1A1 and CYP1B1 mRNA by immunohistochemistry.

Results and Discussion

We will first compare prostate cancer incidence in Phase I participants with population incidence rates reported by the Cancer Registries. We will test the hypothesis that prostate cancer incidence rates (adjusted for age and race) are higher in Vietnam veterans. We will also test whether the prostate cancer incidence rate for African American Vietnam veterans is higher than the corresponding rate for veterans classified as "Other" race. We will then test the more specific hypothesis that exposure to herbicides containing TCDD is associated with an increased risk of prostate cancer. Based on a conservative assumption that only 3% of the population of Vietnam veterans has an elevated level of TCDD, we expect to detect at minimum a 1.7-fold increased risk of prostate cancer, assuming 80% power, a 0.05 significance level, and a 2-sided significance test.

Using Phase II data, we will test whether serum levels of TCDD and its structural analogs are associated with increased prostate cancer risk. We will also test the hypothesis that certain polymorphisms in the *CYP1A1, CYP1A2, CYP1B1,* or *AhR* genes increase the risk of prostate cancer and that observed associations depend on the level of serum TCDD. The two-stage sampling approach should increase the likelihood that sufficient numbers of veterans with the highest TCDD levels will be included. We estimated the lowest detectable odds ratios (LDOR) at allele prevalences ranging from rare (10%, 2.2 LDOR) to common (50%, 1.7 LDOR). With the number of cases and controls set at 225, we assumed a 0.05 significance level, 80% power, and a 2-sided test. Most of the polymorphisms of interest have been reported to have allele prevalences higher than 10%. Using the appropriate sampling fractions and matched analyses, we will also examine gene-environment interactions (i.e., between *CYP/AhR* polymorphisms and TCDD exposure measures).

For specific aim 2, we will test the hypothesis that TCDD levels in exposed veterans are associated with antiestrogenic activity in the prostate. We will test the hypothesis that individuals with the highest serum TCDD levels will have high levels of prostate tissue mRNA expression of P450 genes, but low levels of steroid hormone receptor expression. We will use correlation and linear regression analyses to assess how CYP expression is related to hormone receptor expression. And finally, we will use a variation of the two-sample t-test to examine the relationship between *CYP/AhR* polymorphisms and hormone receptor expression. To test whether the induction of genes by TCDD interferes with estrogen action "downstream" of estrogen receptor-mediated transcription, we will compare the mean estrogen and androgen receptor levels for the group with the "risk allele" (A) with the mean receptor levels for the group with only "wild type" allele (B). The 2-sample t-test will be performed by using the log of the ratio of A and B for each transcript. This transformation is used to ensure that outcomes will be normally distributed. In our experience, the transformation is necessary because the analytical method (i.e., the RT-PCR analysis) cannot reliably detect less than a 2-fold difference in sample values. For specific aim 3, we will test the hypothesis that elevated TCDD levels are associated with increased phenotypic expression of CYP1A1, CYP1A2 or CYP1B1 using correlation and multiple linear regression models. In testing

hypotheses under specific aims 2 and 3, we will also explore the use of multivariate models to allow for the simultaneous assessment of multiple independent and multiple dependent variables.

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