Urinary Glucuronidase and Arylsulfatases in Identical Twins of Bladder Cancer Patients

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ABSTRACT

Studies showing that bladder cancer patients have unusually high levels of urinary β-glucuronidase and arylsulfatases A and B led to the suggestion that these urinary enzymes may participate in bladder cancer etiology. An alternative explanation of the high levels of these urinary enzymes in bladder cancer patients is that the disease itself causes the elevation. Since the levels of these enzymes are genetically determined, measuring these enzymes in healthy identical twins of bladder cancer patients can test whether high enzyme levels occurred prior to bladder cancer. Five healthy identical cotwins of bladder cancer patients, together with matched controls, were measured for urinary β-glucuronidase, arylsulfatases A and B, and two other lysosomal enzymes as controls, α- and β-galactosidases. The mean levels of all five enzymes were not very different in the cotwins and controls, suggesting that high levels of urinary enzymes observed in bladder cancer patients are a consequence of disease rather than occurring prior to disease and contributing to its etiology.

INTRODUCTION

Since the first report that dye workers have an increased incidence of bladder cancer, a large number of aromatic amines and nitroso compounds have been identified or implicated as bladder carcinogens (6). An important step in carcinogenesis by these compounds is conversion to hydroxy derivatives in liver. Hydroxylation occurs on the nitrogen atom in the ring of arylamines (17) and on the alkyl side chains in the α or β positions of nitrosamines (18). Before leaving the liver, the hydroxy derivatives are further metabolized to noncarcinogenic metabolites by conjugation with glucuronic acid or sulfate. Boyland (2) proposed that the bladder was particularly susceptible to the carcinogenic effects of aromatic amines and nitrosamines because incubation of the conjugates in the bladder with urinary glucuronidase and sulfatases resulted in liberation of the active carcinogen. If this sequence of events is correct, then high levels of urinary glucuronidase or arylsulfatases should increase the risk of bladder cancer. Bladder cancer patients do have levels of urinary glucuronidase (3, 4, 9) and arylsulfatases A and B (15) that are well above the normal range. However, high levels of urinary enzymes in patients with bladder cancer do not necessarily mean that those enzymes predispose to bladder cancer; the high levels could result from the disease itself. Previous studies have shown that the levels of urinary glucuronidase were genetically determined (12, 14) and that neither the progeny of bladder cancer patients nor patients judged by their physicians to be symptom free had elevated levels of urinary glucuronidase (13). These data indicate that the high level of urinary glucuronidase probably is not a predisposing factor for bladder cancer but may rather result from the cancer itself.

Measuring first degree relatives is, however, an indirect way of sampling the genotype of bladder cancer patients. If high levels of enzyme were due to a rare recessive allele, then the progeny would be unlikely to be homozygous for that allele and would resemble the normal population. In order to sample the phenotype of bladder cancer patients more directly, we measured urinary enzymes of healthy identical twins of bladder cancer patients together with a set of matched controls.

MATERIALS AND METHODS

Subjects. The subjects were obtained from the National Academy of Sciences-National Research Council twin registry composed of 31,848 World War II male veteran twins. The compilation and maintenance of this registry is described in detail (5, 7). Zygosity of twins, which is estimated to be 95% accurate, was determined by self-report and for many cases confirmed by fingerprint and anthropometric comparisons. The twin registry contained 24 twin pairs for which one twin had bladder cancer, but 12 were dizygotic and excluded from the study. A letter describing the study was sent to the remainder, and 8 agreed to participate. However, 3 were eliminated due to illness or cancer of another type in the cotwin, leaving 5 cotwins in the study. Four were monozygotic, and the fifth (E1) was judged to be monozygotic on the basis of fingerprints and anthropometric measurements, but the surviving twin said that he and his brother were not identical. We used the spouse of each cotwin as a control since the spouse is usually similar in diet, age, race, socioeconomic status, and residence. Use of the spouse has worked well in previous studies from this laboratory (11). In one case, the spouse was not living, so a young woman living in the same household was used as control. All persons who participated were white; the cotwins were male, and the controls were female. Sex does not affect the level of these enzymes (12, 16). Each set of related people was designated by a common letter A to E with the proband being A1, the healthy cotwin A2, and the control A3. Collection of urine has been described previously (13).

Enzyme Assays. Assay of β-glucuronidase, α-galactosidase, β-galactosidase, and creatinine has been described previously (12). Enzyme activities of β-glucuronidase, β-galactosidase, and α-galactosidase are expressed as nmol of product/hr/mg of creatinine. Arylsulfatases A and B were measured using the methods of Baum et al. (1) as modified by...
RESULTS

The characteristics of the probands are given in Table 1. The diagnosis of bladder cancer was obtained from death certificates for Subjects C and E; the other diagnoses were obtained from hospital records. Four of the 5 twins had one parent with cancer, but only one of these was bladder cancer. One twin had a sister with breast cancer. Subject A had kidney cancer 18 years prior to bladder cancer, and the long time lapse makes it likely that they were both primary tumors. Subject C had cancer reported as "unspecified male organs" just 2 years prior to his diagnosis of bladder cancer, so the 2 may have been related. Subject D had bladder cancer at age 41 and a recurrence 10 years later. Subject E had a breast tumor removed at age 24.

The characteristics of the healthy cotwins and the controls are given in Table 2. Except for the one 16-year-old control, used because no spouse was available, the age range was 51 to 60 years, and only one of the 10 currently smoked cigarettes. Arylsulfatase A, ß-galactosidase, and ß-glucuronidase levels in urine show a diurnal variation, so it is important that samples be collected at the same time every day, preferably between 6 and 9 a.m. (10). Although day-to-day variation exists, in part depending on diet, previous studies on lysosomal enzymes have shown that 5 samples are sufficient to characterize individual levels (12).

The values for 5 successive morning urines for the 10 subjects were reviewed, and some data were eliminated from further consideration. Subject B2 and his spouse B3 had extraordinarily low levels (>3 S.D.s from the mean) for all 5 enzymes on the fourth day. Whether this was due to improper handling of urine specimens or due to something in the diet is unknown, but the fourth day samples were eliminated for both subjects. In addition, ß-galactosidase and ß-galactosidase values were eliminated from 6 urine samples due to ß-galactosidase levels less than 1 unit/mg of creatinine and very low ß-galactosidase levels. Previously, it had been shown that these 2 enzymes are inactivated when urine pH was in the alkali range (12). This was thought to occur rarely in normal individuals but apparently occurred more frequently in this set of samples. The problem could be eliminated in the future by including a buffer in the collection bottle.

The means ± S.E.s for the remaining data are shown in Table 3. The enzyme levels from cotwins and controls were compared by paired t tests and by Wilcoxon’s signed rank tests.

DISCUSSION

This study was designed to answer the question whether the high levels of urinary ß-glucuronidase and arylsulfatases A and B observed in bladder cancer patients preceded disease and participated in disease etiology or whether they were a consequence of disease. Previous investigators had reported that bladder cancer patients had glucuronidase levels that were elevated 7-fold (3) or 4.1-fold (4) compared to normal subjects and arylsulfatases A and B levels that were elevated 3.0-fold and 2.1-fold, respectively, compared to normals (15). It is unfortunate that so few identical twins of bladder cancer patients could be identified, but the sample size of 5 cotwins is large enough to rule out a 2- or more fold increase in these enzymes prior to disease (t test). None of the 3 enzymes thought to be involved in bladder cancer etiology showed a 2- or more fold elevation compared to controls suggesting that the elevation of enzyme levels observed in cancer patients results from the disease itself. It is true that the cotwins showed a small elevation in 3 of the 5 lysosomal enzymes that were of borderline statistical significance by the Wilcoxon signed rank test. Of the 3 enzymes thought to be involved in bladder cancer etiology because they have a role in metabolism of bladder cancer carcinogens, glucuronidase, arylsulfatase A, and arylsulfatase B had increases of 28, 37, and 0% compared to the increases of 4-fold, 3-fold, and 2-fold reported in the literature for bladder cancer patients. Furthermore, ß-galactosidase and ß-galactosidase, which were included as controls because they have no known role in metabolism of bladder carcinogens, showed 75 and 48% increases that were also of statistical significance. Thus, we conclude that these small increases in both test and control enzymes, although statistically significant by one test, were not of biological significance to the hypothesis being tested.

This study only eliminates genetically determined levels of urinary enzymes as a predisposing element in bladder cancer; it
This is not surprising since urinary lysosomal enzymes have cancer itself causes the high levels of urinary lysosomal enzymes. The most probable interpretation of all data is that bladder patients, although currently, no data exist to support this hypothesis. This does not rule out the possibility that some environmental agent cautious concerning hypotheses which arise from enzyme measurement. It may be that these enzymes would be useful in monitoring bladder cancer patients in remission as an indication that disease is recurring. The concept that individuals are genetically susceptible to cancer due to variations in levels of carcinogen-metabolizing enzymes is an attractive one. However, one must be very cautious concerning hypotheses which arise from enzyme measurements on cancer patients. These enzyme levels are likely to be altered by disease. In this respect, the proposal that genetically determined urinary enzyme levels were important in bladder cancer etiology parallels the similar suggestion that aryl hydrocarbon hydroxylase inducibility differed in a population of lung cancer patients (8). However, neither the progeny of patients nor cured lung cancer patients showed any difference in aryl hydrocarbon hydroxylase inducibility from the normal population (11, 19).

No doubt genetic polymorphisms in carcinogen-metabolizing enzymes alter susceptibility to particular types of cancer. However, each suggested hypothesis that derives from data on sick persons should immediately be tested by first determining if population variation is genetic in origin and then measuring the enzymes in close relatives of the patient; progeny; siblings; parents; or twins.

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