Urinary Excretion of Modified Nucleosides in Patients with Malignant Mesothelioma

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ABSTRACT

Transfer RNA is the most complex biomacromolecule in both structure and function. The complexity of its structure is caused by a large variety of enzymes which add modifying groups to the four bases after the primary synthesis. The most abundant of these enzymes are the transfer RNA methylases, which add methyl groups at various positions in the macromolecule. These methylating enzymes were found to be, without exception, aberrantly hyperactive in every malignant tumor examined.

In turn, every malignant tumor contains a few transfer RNAs that are different in structure from the transfer RNAs in the normal tissue. Again, there is no exception. These are the first qualitatively different biochemical components of every malignant cell, not more or less but different transfer RNAs.

The late Alexander Gutman observed that cancer patients excrete in their urine elevated levels of certain methylated bases. From the structure of these bases and our knowledge of their method of synthesis, it became apparent that most of them come from the breakdown of transfer RNA. Their elevation in the urine stems from an extraordinarily high rate of turnover of transfer RNAs in tumor tissue. Highly sophisticated, sensitive methods of analysis were developed for the determination of the modified nucleosides in the urine of cancer patients. When related to the creatinine level of the urine, some of the modified nucleosides and products derived from them were elevated in a large variety of tumors. Perhaps more importantly, it was found that these elevated levels return to normal after effective chemotherapy. Thus, these markers may also be useful in monitoring the effectiveness of therapy. We report here initial studies on the detection of cancer in asbestos workers and possible premalignant conditions in workers with asbestosis.

INTRODUCTION

It has been known for some time that cancer patients excrete in their urine elevated levels of certain methylated bases. From the structure of these bases and our knowledge of their method of synthesis, it became apparent that most of them come from the breakdown of transfer RNA. From these studies, it became evident that the excretion level among normal subjects has a very narrow range when related to body weight. The level of excretion of another product in the urine, creatinine, is also closely related to body mass. Therefore, we undertook extensive studies to determine whether the level of excretion of modified nucleosides could be related to the creatinine level of random samples of urine. From these studies, it became apparent that the ratio of modified nucleosides to creatinine in random samples was the same as that in total urine excreted in 24 hr (7). These findings imply that the excretion is not episodic and also that there must be a stringent control over the turnover of transfer RNA.

Significant elevations (more than 2 S.D.s) were found in a large number of patients with different oncological problems. However, the elevations and the variety of markers are not the same in all patients with different types of tumor burden (20). For example, excretion levels were very high in patients with Burkitt's lymphoma, a tumor with a high proliferative growth factor (21). On the other hand, patients with breast cancer, a tumor with a high proportion of cells in G0, have a much lower frequency and degree of nucleoside elevation (23).

In early feasibility studies of the usefulness of markers, the urine of 62 patients with diseases other than cancer was analyzed (22). The diseases were: hepatitis; chronic obstructive pulmonary disease; cirrhosis; acute cholecystitis; acute pancreatitis; regional enteritis; rheumatoid arthritis; ulcerative colitis; and psoriasis. In the urine of certain of these patients, there were some elevations, but these were low compared to the values in patients with malignant diseases. After effective chemotherapy, the marker levels in subjects with Burkitt's lymphoma return to normal within 1 week to 10 days. Since this observation, similar findings were reported with childhood leukemia, Hodgkin's as well as some undifferentiated lymphomas, choriocarcinoma, and adult leukemia. All of these findings and specific references were reported in 2 recent review articles (3, 19).

Asbestos-associated diseases constitute a major public health problem in the United States (15). The increased use of asbestos in a variety of industrial processes during the first half of this century has resulted in exposure for a large number of individuals. It is estimated that more than 27 million people may have experienced significant direct or indirect occupational exposure to asbestos from 1940 to 1979 in a variety of industries, including shipbuilding and ship repair, construction, transportation, power production and utilities, chemical manufacturing and refining, brake maintenance, and railroad work. It is estimated that more than 20 million exposed individuals were alive at the beginning of 1982 (13).

Both nonmalignant and malignant diseases have been shown
to be associated with inhalation of asbestos fibers. In the 1930s, a high proportion of abnormal chest X-rays suggestive of asbestosis, i.e., pulmonary interstitial fibrosis, was reported in asbestos-exposed populations (10, 12); and asbestosis is still the most common disease affecting individuals exposed to asbestos in the past. In 1960, following early reports indicating an association between asbestos exposure and lung cancer (5, 11), evidence appeared indicating that previous asbestos exposure was also related to the development of malignant pleural mesothelioma (24). At that time, this disease was very rare in the general population (without a history of asbestos exposure) and has remained so until today. In contrast, it is a common neoplastic disease among asbestos workers. Epidemiological investigations have reported that almost 10% of all deaths among such individuals were due to malignant mesothelioma (14, 16, 18). Thus, asbestos is closely related etiologically to mesothelioma (4). It should be emphasized that cigarette smoking acts synergistically with asbestos in the development of lung cancer (18) but that mesothelioma develops independently of cigarette smoking.

One of the characteristics of asbestos-related diseases is the long period of clinical latency between first onset of exposure and the development of the disease. For mesothelioma, this latency period is commonly 30 to 40 years (18). Mesothelioma is usually a highly malignant tumor, resistant to most modalities of therapy including chemotherapy, radiation, and surgery. Despite some therapeutic advances (1), the prognosis is poor. This is so even after diagnosis has been obtained as early as possible by currently available diagnostic means, including chest X-ray, computerized axial tomography scan, gallium scan, biopsy, and histochemical analysis. Survival is usually less than 2 years. Because of this situation, there is an urgent need for developing new diagnostic methods which could assist in the identification of pathophysiological changes at a stage when early intervention might be of added advantage. We report observations of the urinary excretion patterns of modified nucleosides in patients with malignant mesothelioma as a possible approach for the early detection of this disease.

MATERIALS AND METHODS

Eight individuals with malignant mesothelioma were examined at The Mount Sinai Medical Center. Seven were males; all but one had malignant pleural mesothelioma; one had peritoneal mesothelioma. A summary of pertinent anamnestic and clinical data is presented in Table 2. In addition, urine samples of 13 male workers with long-term exposure to asbestos and with roentgenologically verified asbestosis, but without clinical evidence of malignant disease, were analyzed. Anamnestic and clinical information on these patients is presented in Table 3.

Random samples of urine were collected in sterile plastic containers and kept at −15° to −20° without any preservative. No special requirements as to diet or medications were imposed upon the patients. The samples were packed with dry ice in Styrofoam boxes and shipped by air express to the AMC Cancer Research Center in Denver, where they were received in a frozen state. On arrival, they were logged and stored at −80° until analysis. The compounds determined were pseudouridine, 1-methyladenosine, 2-pyridone-5-carboxamide-N'-ribosyladenosine, 1-methylcytosine, 1-methylguanosine, N²-methylguanosine, N²,N⁴-dimethylguanosine, and BAIB. The analytical methods for the quantitation of modified nucleosides (6, 9) and BAIB (8) have been described in detail. Urinary nucleosides and total BAIB (free and conjugated) were determined in duplicate aliquots of the urine. Duplicate analyses of those samples with more than 5% variation were repeated. In Tables 2 and 3, averaged values were recorded. Creatinine was measured in 3 different dilutions of the urine by a creatinine analyzer (Beckman Instruments, Inc.). The rate of reaction was measured 25.6 sec after sample introduction to minimize interference due to the presence of nonspecific chromogens. All samples were coded and analyzed in a double blind manner, with neither the technicians nor the investigators able to identify the origin of the samples prior to analysis. After analysis, the clinical investigators and laboratory investigators exchanged pertinent information.

RESULTS

Normal values for the excretion of nucleosides were determined from earlier studies (3, 20) consisting of males ranging in age from 19 to 58 years old and females 20 to 81 years old (Table 1). The range of excretion of these markers in normal subjects is very narrow relative to creatinine content. There were small variations with respect to age and diet, but in general, urinary nucleosides were higher in females than in males. The range of daily excretion levels in the same subjects was similar (3, 19). Levels of urinary nucleosides in persons with diagnosed mesothelioma are shown in Table 2. (Marker levels elevated 2 S.D.s above the normal mean value are considered abnormal.) Several nucleosides were elevated, and the excretion pattern was characteristic of cancer in all patients. Pseudouridine was elevated in all patients. The nucleoside excretion patterns of 13 asbestos workers are shown in Table 3. Ten individuals had elevated levels of nucleosides, while the excretion patterns were considered "borderline" in 3 cases. In these, elevated excretion of pseudouridine was the principal abnormality.

DISCUSSION

It is predicted that the incidence rates of asbestos-related neoplastic diseases will increase during the next 2 decades, even in the absence of further exposure (13), and will then gradually decrease. It will thus pose a serious public health problem in the United States for the foreseeable future. In some groups of asbestos workers, more than 40% of deaths have been due to cancer (16, 18).

One of the most characteristic features of asbestos-associated diseases is the long period of clinical latency from the time of onset of exposure to the appearance of clinical symptoms (17). Thus far, there is little information about the biological events occurring during this latent period.

At the present time, the diagnosis of mesothelioma, often very
### Table 2

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Note: *p* < 0.05

### Table 3

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complex histopathologically, is almost exclusively made at a stage when therapeutic intervention is of limited value in terms of lengthened survival. The data presented here on patterns of urinary excretion of modified nucleosides in both patients with mesothelioma and those with asbestosis, but without this neoplasm, indicate that mesothelioma produces elevated excretion of nucleosides and that this may be an additional diagnostic tool. No false-negative results were observed in this small group of patients with mesothelioma, and the laboratory investigators were able to identify all 8 as “cancers” on the basis of the quantitation of nucleosides. Although no clinical “staging” of the clinical latency. They will be followed prospectively. Such studies of lengthened survival. The data presented here on patterns of

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REFERENCES

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