ANTIBODIES AGAINST FREE RADICAL MODIFIED DNA IN CANCER PATIENTS

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ABSTRACT

In this study we have modified DNA by exposing it to ultraviolet light in the presence of hydrogen peroxide. The modified DNA was probed for binding to the antibodies present in the sera of patients suffering from various types of cancer. Higher recognition of modified DNA, as compared to native DNA, by antibodies from cancer patients has got far reaching significance.

KEY WORDS : Reactive oxygen species, Cancer, DNA damage.

INTRODUCTION

Any substance that reacts with DNA is potentially carcinogenic. Exposure of DNA to superoxide generating systems causes extensive strand breakage and degradation of deoxyribose (1), probably due to formation of hydroxyl radicals. Oxygen free radicals, especially hydroxyl radicals generated during cellular metabolism, have been known to cause DNA damage and their relevance in carcinogenesis is well documented (2-4). Radiations are a major source of reactive oxygen radicals (ROS) (5). Ultraviolet rays damage DNA indirectly through photosensitised reaction mediated mainly by singlet oxygen which generates purine base modification and has been implicated in carcinogenesis (6). It has been observed to induce neoplasia in both humans and experimental animals (7, 8).

In this study native calf thymus DNA was modified by reactive oxygen species generated by UV irradiation of H₂O₂ (9). After characterization, antibodies against modified DNA in the sera of cancer

patients were detected and quantitated by ELISA (9). Circulating anti-DNA antibodies in cancer patients had also been detected by ELISA using purified calf thymus DNA as antigen. Specificity and cross reactivity of these antibodies were also ascertained by competition- inhibition ELISA (10).

MATERIALS AND METHODS

Sera

Present study was conducted on the sera of 30 histologically proven cancer patients. Serum samples were also analysed from 20 healthy age and sex matched controls. Sera were decomplexed in a water bath maintained at 56 °C for 30 min. and stored at -20 °C with 0.2 % sodium azide as preservative.

Chemicals

Calf thymus DNA, anti-human IgG alkaline phosphatase conjugate, S1 nuclease were from Sigma chemicals company, USA. Sepharose 4B was from Pharmacia fine chemicals, Sweden and ELISA plates were obtained from Nunc, Denmark. All other chemicals used were of highest grade available.

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Laboratory methods

Commercially available, highly polymerised calf thymus DNA was purified free of protein and single stranded regions (11). Colorimetric estimation of DNA was carried out according to the method of Burton (12) using diphenylamine reagent. Protein was estimated by the method of Lowry et. al. (13). ROS modification of DNA was done according to the methods of Ara et. al. (9) using 254 nm ultraviolet light. The concentration of hydrogen peroxide was determined by titration with sodium thiosulphate (14). Determination of melting temperature was done by determining mid point melting temperature (T_m) on a Shimadzu UV-240 Spectrophotometer coupled with a temperature programmer and controller (15). For detection and quantitation of antibodies, ELISA was performed as per the method of Aoutsuka et. al. (16) with a slight modification (17). The antigen binding specificity of antibodies was defined by competition-inhibition experiments (18).

RESULTS

Characterization of ROS-DNA

The ROS modified DNA was characterised by the ultraviolet absorption spectroscopy and thermal denaturation studies. Modified DNA showed hyperchromicity at 260 nm and decrease in melting temperature (T_m), as compared to unmodified DNA control of same concentration. These results are consistent with the earlier recorded observations (19).

Detection of anti-ROS-DNA antibodies in cancer patients

A total of 30 patients of cancer were the subjects of the present study. They were suffering from cancer of female genital tract e.g., vagina, cervix & ovary ($n = 7$); breast cancer ($n = 4$); oral cancer ($n = 8$); laryngeal cancer ($n = 4$) and miscellaneous ($n = 7$)

which included cancers of gastrointestinal tract, bone, lung and neurogenic sarcoma. All the patients were in the advanced (clinically second or third) stage of the disease. Twenty age and sex matched healthy persons served as controls in this study.

Binding characteristics of antibodies from the sera of cancer patients was analysed by ELISA. Figures 1-3 show the binding of various cancer sera with ROS-DNA and native DNA. Out of thirty sera from five groups, twenty nine showed high binding to ROS-DNA as compared to native DNA. The only exception being a lone case of carcinoma lip (sample 6, fig. 1) which had a lower binding with ROS-DNA as compared to native DNA but still higher than the binding of ROS-DNA with healthy adult human sera. On the other hand, sera from normal healthy controls showed negligible binding with ROS-DNA, while the binding of these control sera samples to native DNA was almost comparable to the native polymer binding of sera from cancer patients.

Figure 1. Binding of antibodies from the sera of larynx and oral cancer patients [1-12] to native (-□-) and ROS-modified (-■-) DNA. N represents normal human sera.

Specificity of antibodies found in the sera of cancer patients towards ROS-DNA was checked in competition assay. Out of 30 sera that were studied in direct binding ELISA, seven serum samples giving appreciably higher binding with ROS-DNA were used in the inhibition ELISA. The depletion in the antibody activity ranged from 24.2% to 41.0% for ROS-DNA. On the other hand, native DNA could produce an inhibition only from 6.5% to 22.0% under identical experimental conditions (Table 1).

Figure 2. Binding of antibodies from the sera of female genital organ and breast cancer patients [1-11] to native (-□-) and ROS-modified (-■-) DNA. N represents normal human sera.

Figure 3. Binding of antibodies from sera of cancer (miscellaneous) patients [1-7] to native (-□-) and ROS-modified DNA (-■-). N represents normal human sera.

DISCUSSION

The damaging effect of ROS, like superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\cdot$) on nucleic acids, proteins and lipids has generated considerable interest (20-21). These radicals modify DNA at various sites that include base damage and cross-linking between cytosine and tyrosine in nucleohistone (22). Cellular

Table 1. Competition-inhibition of antibodies from the sera of various cancer patients

Serum Classification	Maximum Percent inhibition with DNA	Maximum Percent inhibition with ROS-DNA
Ovarian Cancer	20.7	32.0
Breast	16.2	37.5
Carcinoma Cheek	10.2	27.1
Neurogenic Sarcoma	22.0	40.2
Maxillary Carcinoma	17.1	40.2
Alveolus Carcinoma	7.8	41.0
Carcinoma nose	6.5	24.2

DNA is damaged by the oxygen free radicals generated during cellular respiration; cell injury, phagocytosis and exposure to environmental oxidants (23). The resultant damage to DNA bases may be significant source of mutations that lead to cancer and other human pathologies (24-25). The oxidative stress to genetic material has been proposed to be initiating step in diseases like cancer, ageing and autoimmune disorders. Continuous endogenous damage to cellular DNA by free radicals and accumulation of such damage has been suggested to significantly contribute to carcinogenesis in humans (26).

The recognition of the ROS-DNA by the antibodies from cancer patients has got far reaching significance. The results of ELISA wherein increased absorbance was observed at 410 nm for the plate coated with ROS-DNA compared to native DNA coated plate signify stronger binding of the ROS-DNA to the antibodies present in the sera of cancer patients. Specific binding of the antibodies present in the sera of cancer patients to ROS-DNA was ascertained in

the inhibition-ELISA. While native DNA inhibited the antibody activity by 6.5 to 22.0 %, ROS-DNA depleted the antibody activity by 24.2 to 41.0 %. This signifies high specificity of cancer antibodies towards ROS-DNA. Recognition of ROS-DNA by antibodies present in cancer patients is significant because reactive oxygen species, formed as a result of exposure to various radiations, chemicals, dietary contaminants and other factors, can induce modifications of genomic DNA. This alteration / mutation if not edited can progress through replication and lead to cancer besides producing altered gene products. The study successfully establishes the presence of autoantibodies against ROS-DNA in the sera of diversified cancer patients. The data, though preliminary, may go a long way in establishing these antibodies as biological marker for cancer diagnosis as well as to track the prognosis during the course of therapy. However, binding of antibodies, present in the sera of patients suffering from other diseases, to ROS-DNA will be undertaken to establish the exact epitopes involved in the induction of these antibodies in cancer. This may help in defining absolute specificity of these antibodies for cancer.

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