Europ. J. Cancer Vol. 17, No. 5, pp. 533–538, 1981
Printed in Great Britain

0014-2964/81/050533-06$02.00/0

A Comparison of DNA and DNA-Binding Protein Levels in Malignant Disease*

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Abstract—*Measurements of serum concentrations of DNA and the DNA-binding protein C3DP by radioimmunoassay showed that the levels of both substances tend to increase in cancer patients during active malignant disease. In most cases, the levels returned to normal during chemotherapy-induced remission; however, the changes in concentration for DNA and C3DP did not occur simultaneously, and no correlation was found between their levels. The sera of cancer patients contained a strong inhibitor of DNAse. We examined the possibility that C3DP may have such an inhibitory effect by binding to DNA and preventing the action of DNAse. The enzyme was fully active in the presence of purified C3DP, indicating that the DNAse inhibitor in cancer serum was a substance other than C3DP. Although the relationship between DNA and the DNA-binding protein remains unknown, their measurement may have diagnostic and prognostic value.*

INTRODUCTION

In a recent investigation, we found that cancer patients have increased levels of DNA in the circulation. The mean concentration of DNA in the sera of 173 patients was 180 ± 38 ng/ml, whereas 61 normal healthy controls showed a value of 13 ± 3 ng/ml [1]. A separate investigation revealed that cancer patients also had abnormally high levels of the DNA-binding protein, C3DP, derived from complement component C3. In the sera of 47 patients with active carcinomas, a mean concentration of 242 μg/ml of C3DP was found, while the mean for 31 normal controls was 83 μg/ml [2]. The levels of both substances increased during active disease, especially in the presence of metastases, and decreased as a result of successful treatment and remission [1, 3]. No specificity was found for the histological type, size, or location of the tumor. In view of the possible diagnostic application of these measurements, as well as a means for following treatment [1–3], we thought that it was particularly relevant to determine the levels of both DNA and C3DP in the same patients, since by definition there may be an interaction between the two substances.

Accepted 3 November 1980.
*Supported by grant PDT-163 from the American Cancer Society, Public Health Service grant BRSG-N-1018, and Postdoctoral Research Fellowship GM-05148 awarded by the National Institute of General Medical Sciences.

MATERIALS AND METHODS

Patients and sera

Serial serum samples were obtained from seven cancer patients (four with breast carcinoma, one Hodgkin's lymphoma, one stomach carcinoma, and one colon carcinoma) during the course of chemotherapeutic management, for periods of up to 10 months. Blood samples were obtained by venipuncture, and sera were separated after clotting at 4°C, frozen and kept at −20°C until use. A total of 104 samples were collected and assayed for both C3DP and DNA.

Radioimmunoassays

C3DP was determined by radioimmunoassay as described previously [3]. Values between 50 and 150 μg/ml were considered to be within the normal range, whereas above 150 μg/ml they were considered elevated [2]. DNA was tested by radioimmunoassay, using *E. coli* ^{125}I-iododeoxyuridine-labeled DNA (^{125}IUdR-DNA) and antibody from the serum of a patient with systemic lupus erythematosus [1, 4]. The limit of sensitivity of the assay is 25 ng/ml, and the presence of DNA can be determined in biological fluids such as

serum, plasma and synovial fluid, without extraction and purification [1, 4]. In the serum of normal healthy individuals, the DNA concentration is 0–50 ng/ml. In patients with some inflammatory conditions, such as rheumatoid arthritis [5], levels of up to 100 or 150 ng/ml can be seen. In cancer patients, concentrations of up to 5000–10,000 ng/ml have been measured, although in some cases normal values of 0–50 ng/ml have been found. Thus, normal levels cannot rule out pathological conditions, whereas concentrations above 50–100 ng/ml are usually associated with disease [1].

Isolation of DNA-binding C3DP

A DNA-binding form of C3DP was isolated by a modification of the previously described method for C3DP purification [6]. The buffers (without 2-mercaptoethanol) used for washing and eluting the DNA-cellulose column were 10 mM potassium phosphate buffer (50 mM NaCl, 1 mM EDTA, pH 6.8) and 10 mM potassium phosphate buffer (50 mM $NaClO_4$, 1 mM EDTA, pH 6.8), respectively. The ammonium sulfate precipitation has been omitted. Chromatographic fractionations on the DEAE-cellulose and Sephadex G-150 columns were performed as previously described. Protein isolated in this manner retained its full capacity for DNA-binding [6].

DNAse and DNAse-inhibitor assay

The possible inhibitory effect of either serum of cancer patients or purified C3DP on DNAse was tested. The reaction mixture contained the following reagents: purified DNAse 1 (Worthington, sp. act. 2500–2900 units/mg) dissolved at a concentration of 50 ng/ml in 0.06 M Tris-chloride buffer (pH 7.4) containing 5 mM $MgCl_2$ and 1 mM $CaCl_2$, ^{125}IUdR-labeled DNA diluted in the same buffer at a concentration of 4 μg/ml [4, 7], and the test serum.

The following controls were used: enzyme alone—10 μl of ^{125}IUdR-DNA, 200 μl of Tris-Cl buffer with metals, and 10 μl of DNAse; serum alone—the DNAse was omitted and 10 μl of serum were added instead; full reaction mixture—both enzyme and test serum were present. The mixtures were assembled in an ice water bath, mixed and incubated at 37°C for 0–5 hr, and chilled in ice for 5 min. Then 10 μl of normal human serum were added to the control mixture ("enzyme alone") to act as carrier for the precipitation step. Two hundred microliters of 10% TCA were added, mixed, and let stand for 15 min. The precipitate was removed by centrifugation at 5000 rev/min for 10 min; the supernatant was aspirated and counted in a gamma counter for ^{125}I radioactivity. At zero time, 97–100% of the DNA was acid precipitable. After background correction, the fraction remaining undegraded was calculated and plotted versus time. The control mixture (enzyme alone) was taken as 100% activity and the percentage inhibition was calculated from the full reaction mixture (enzyme + serum), usually at the 2 hr point.

RESULTS

Distribution of C3DP and DNA levels

The concentrations of C3DP in 104 samples from seven patients showed a range of 60–300 μg/ml and a mean ± S.E.M. of 140 ± 6 μg/ml. The values for DNA had a range of 0–4000 ng/ml with a mean ± S.E.M. of 248 ± 58 ng/ml. The distribution of these levels is shown in Fig. 1. The two solid lines represent the upper limits of normal values [1, 2]. The graph is divided into four sections by the two lines: section B includes the samples with normal levels of both C3DP and DNA; section C contains the samples with elevated C3DP and DNA; section A represents the samples with normal DNA levels and high C3DP levels; and section D includes the samples with normal C3DP values and abnor-

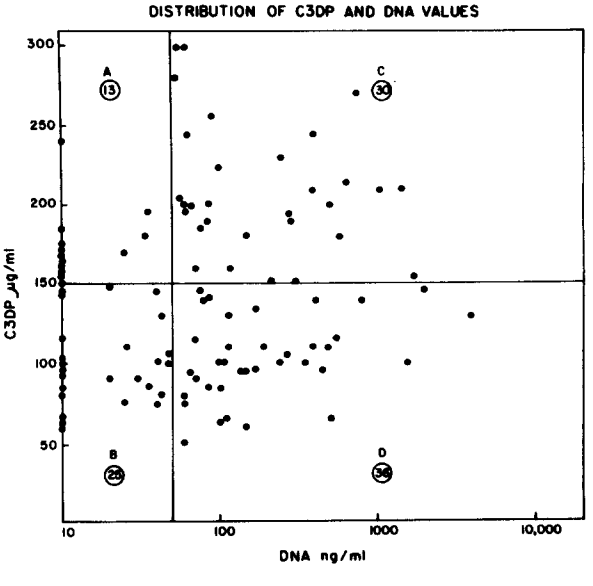

Fig. 1. Distribution of C3DP and DNA levels in 104 samples from seven patients (see Materials and Methods). The solid horizontal line parallel to the abscissa represents the upper limit of normal values for C3DP; the line parallel to the ordinate indicates the upper normal limit of normal values for DNA. The circled numbers indicate the number of samples in each of the four resulting sections (A, B, C, D).

mally high DNA values. The number of samples for which the two tests are in agreement (sections B and C, 55 samples or 53%) is slightly higher than the number for which they disagree (sections A and D, 49 samples or 47%). The correlation coefficient calculated for the two parameters is 0.01.

C3DP and DNA levels during cancer treatment

The levels of the two substances in these patients throughout different regimens of chemotherapy showed that the individual profiles of C3DP and DNA levels were as illustrated below. In some patients, the increase of DNA concentration in the serum preceded a relapse and then fell during progress in tumor growth (Fig. 2). In other patients, both DNA and C3DP decreased during remission, and increased subsequently reflecting a relapse (Fig. 3). Additional information concerning the clinical course of disease in these patients has been previously reported [3].

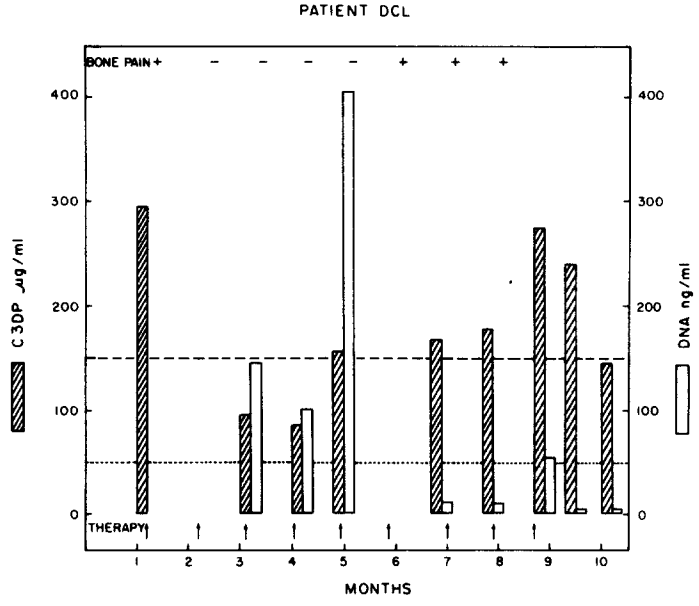

Fig. 2. Levels of C3DP and DNA, and the effect of therapy in patient DCL with metastatic breast carcinoma. Chemotherapy consisted of cytoxan, methotrexate and 5-fluorouracil (see ref. 3).

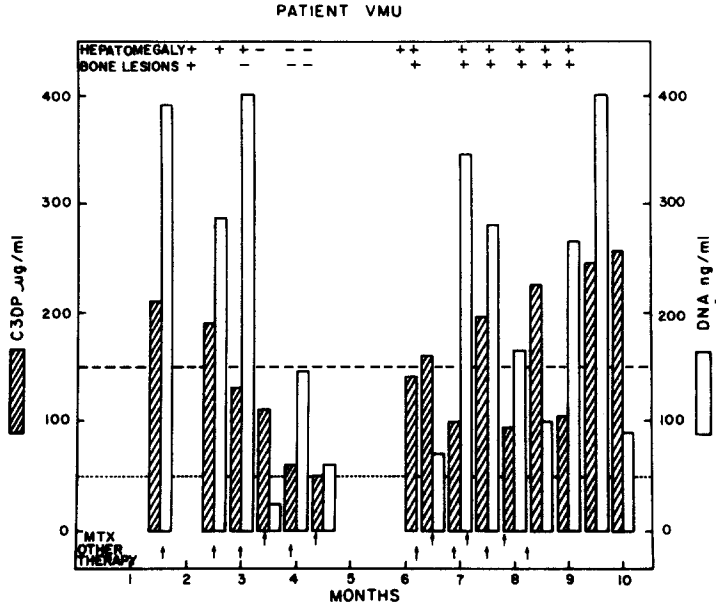

Fig. 3. Levels of C3DP and DNA in patient VMU with metastatic breast carcinoma, receiving cytoxan, methotrexate and 5-fluorouracil (see ref. 3).

Determination of DNA in the presence of C3DP

Since a possible interference could result from binding of C3DP to DNA (either cold or labeled), thus altering the amount of DNA available for binding to the anti-DNA antibody, the effect of C3DP on the radioimmunoassay of DNA was tested. In order to quantitate the effect, unlabeled DNA at a concentration of 200 ng/ml and C3DP at a concentration of 710 μg/ml were added to the reaction mixture. This concentration was chosen because it is in the most sensitive part of the calibration curve [4]. Under these conditions, an apparent concentration of 101 ±7 ng/ml was calculated (Table 1). We conclude that in the presence of C3DP at 710 μg/ml, the DNA levels in the patients may be underestimated by as much as 50%. It should be pointed out, however, that this concentration of C3DP is substantially higher than that found in sera from cancer patients examined so far (maximum of 300 μg/ml).

Table 1. DNA radioimmunoassay in the presence of C3DP

	^{125}I-DNA binding (%)*	
	No C3DP	+C3DP (710 μg/ml)
Non-specific binding (no antibody)	4.0±0.1	6.3±0.3
Zero control (no cold DNA)	35.0±0.4	38.2±0.7
Added cold DNA (200 ng/ml)†	14.0±0.5	18.3±0.6 (eq. 101±7 ng/ml) of DNA

*Total radioactivity, 5795 cpm of ^{125}I-DNA. Results are the mean ±S.E.M. of two assays in triplicate, corrected for non-specific binding, and represent net binding of ^{125}I-DNA to antibody or C3DP.
†Reproducibility is within 8% at this range of the calibration curve [4].

DNAse activity in the presence of C3DP

A possible cause for the accumulation of serum DNA in cancer patients may be lower DNAse activity in their serum [8]. Such low activity may be due to the presence of a DNAse inhibitor [9, 10]. The possibility also exists that DNA binding by C3DP may render this substrate either inaccessible or unsusceptible to the enzyme. To test this possibility, we measured the activity of pure DNAse on DNA in the presence of 710 μg/ml of purified C3DP. The results (Fig. 4) showed that the enzyme was fully active in the presence of C3DP. In contrast, when 10 μl of serum from a cancer patient was added to the reaction mixture, the DNAse activity was inhibited completely. The reason for the apparent activity detected in the later stages of the reaction is unknown at present. For comparison, the effect of normal serum (to which unlabeled DNA was added to a concentration of 300 ng/ml, as found in the cancer serum) was also tested (Fig. 4). We found some inhibitory effect, much less pronounced than with cancer serum. We conclude that (a) C3DP does not inhibit the DNAse degradation of DNA, and (b) cancer serum contains an inhibitor of DNAse.

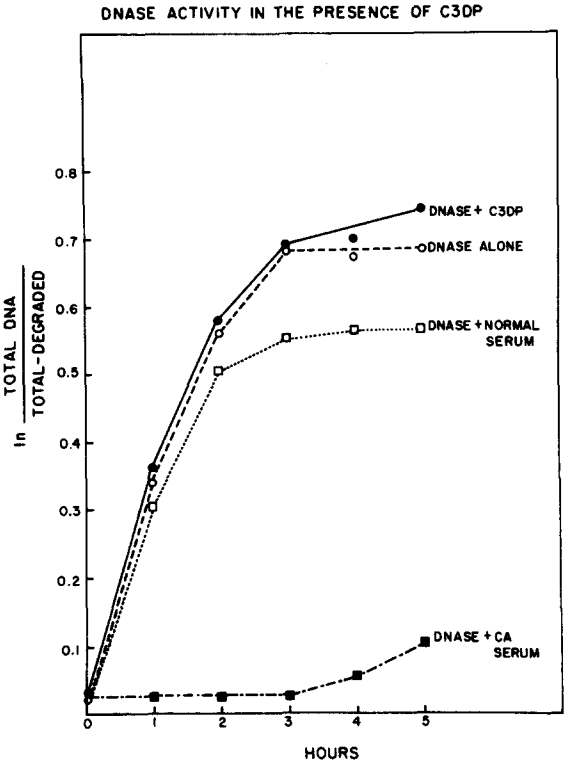

Fig. 4. DNAse activity in the presence of C3DP. Degradation of ^{125}IUdR-labeled DNA by purified DNAse I in the absence and presence of C3DP at 710 μg/ml, and in the presence of 10 μl of normal or cancer serum, both containing 300 ng/ml of unlabeled DNA.

We then examined the possibility that binding of C3DP to DNA may be either delayed or prevented in the presence of DNAse. Thus C3DP and DNA were preincubated prior to the addition of the enzyme. Preincubation of DNAse alone, and DNAse + C3DP, served as control. The results (Fig. 5) showed no effect on the degradation of DNA under these conditions. We conclude that the inhibitor of DNAse in cancer serum was due to a substance other than C3DP.

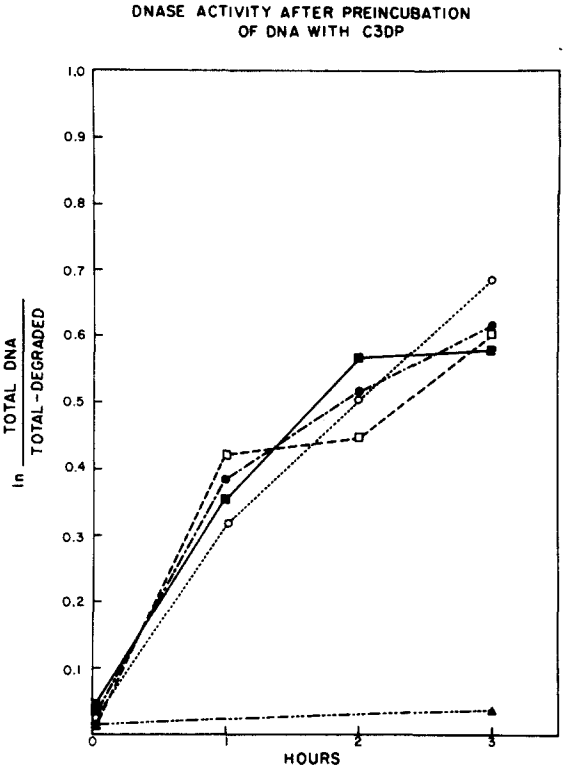

Fig. 5. DNAse activity after preincubation of DNA with C3DP. ^{125}IUdR-labeled DNA was preincubated with C3DP (710 μg/ml) at 37°C for 1 hr, then DNAse was added (closed circles). DNAse was preincubated with C3DP under the same conditions, then ^{125}IUdR-DNA was added (closed squares). Controls: DNAse without preincubation (open circles); DNAse preincubated alone (open squares); DNAse activity in the presence of 10 μl of cancer serum (closed triangles).

DISCUSSION

Several proteins have been detected in the circulation of cancer patients at abnormally high concentrations: carcinoembryonic antigen [11], α-fetoprotein [12], placental proteins [13], C3DP [2] and a fibronectin-like fraction [14]. These substances are either absent or at significantly lower concentrations in normal healthy individuals. One of the properties of the last two proteins is their ability to bind to DNA. This fact, and the high levels of DNA found in the circulation of patients with malignancy, prompted us to investigate their relationship. The levels of C3DP and DNA in the circulation of cancer patients tend to increase during active growth and spread of the tumor, and decrease during remission [1, 2]. This general trend was observed in the present study, although the concentration changes did not occur *simultaneously*. The lack of temporal correlation suggests that the two substances may reflect different aspects of the neoplastic process. Since both substances are associated with active disease, the measurement of their levels may have diagnostic and prognostic relevance.

The high levels of circulating DNA in malignancy may result from abnormally low DNAse activity, assuming that DNA is degraded by DNAse and cleared from the circulation in healthy humans. We reasoned that such low activity could be due to the interaction of DNA-binding proteins with DNA, thus rendering the substrate inaccessible or unsuitable for the enzyme. The results of the present work showed that DNA is fully active in the presence of C3DP, even when that protein is preincubated with DNA prior to the addition of the enzyme. Further, addition of 10 μl of serum from cancer patients totally inhibited the purified DNAse, whereas normal serum had a much smaller effect. We conclude that the mechanism for the accumulation of DNA in the circulation may result from inhibition of DNAse activity, mediated by an inhibitor other than C3DP. A naturally occurring inhibitor of DNAse has been isolated from both normal and malignant cells and identified as actin [15, 16]. This protein has been shown to form specific 1 : 1 mole complexes with DNAse [17]. Experiments are in progress to determine whether the inhibitor in the serum of cancer patients is identical to actin.

Acknowledgments—We thank R. Adler for excellent technical assistance and B. Graubard for expert help in the preparation of the manuscript.

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