Acta Oncologica 29 (1990) Fasc. 2

FROM THE TOXICOLOGIC AND CANCEROLOGIC BIOCHEMISTRY UNIT, UCL 7369, AND THE CANCEROLOGIC UNIT, UCL 5470, MEDICAL FACULTY, UNIVERSITY OF LOUVAIN, BRUSSELS, BELGIUM.

PREDICTIVE VALUE OF SERUM ALKALINE DNase ACTIVITY VARIATIONS IN TREATMENT OF HEAD AND NECK CANCER

A. ECONOMIDOU-KARAOGLOU, M. OPSOMER, M. LANS, H. S. TAPER, C. DECKERS and M. B. ROBERFROID

Abstract

The aim of this study was to evaluate the variation in serum alkaline DNase activity (SADA) as a means of therapeutic monitoring in patients with head and neck cancer. Blood samples from 40 patients were collected before, during, and some weeks up to months after therapy. A decrease in SADA during treatment was usually associated with a primary clinical response, while no decrease indicated non-response to therapy. In patients with complete tumor regression the initial decrease of SADA was usually followed by an increase exceeding the initial level. A similar increase was not observed in patients with tumor progression.

Key words: Head and neck cancer, tumor marker, DNase I, treatment response.

The present study proposes that variations in serum alkaline DNase activity (SADA) can be utilized for the early prediction of response to tumor therapy.

Previously published histochemical observations have indicated that effective anticancer treatment induces typical variations in alkaline DNase activity. These studies have shown that the activity of this enzyme is low or absent in experimental and human malignant tumors (1–3). After treatment, a histochemically detectable increase in alkaline DNase activity has been observed in tumors sensitive to therapy but as a rule not in the activity of resistant tumors (4).

More recent clinical studies of different malignancies (5–8) have shown characteristic SADA variations after efficient treatment. SADA also appears to be promising for early detection of occult recurrence in acute non-lymphoblastic leukemia.

The present study analyzes the value of SADA as a clinical marker in patients with head and neck cancer.

Material and Methods

The study included 40 patients from three hospitals in Belgium, at different stages of disease (Table). The diagnoses were based on histologic and/or cytologic examinations and the patients were treated with chemotherapy and/or radiotherapy.

The patients were treated with one of the following protocols. The first was 5-fluorouracil and and cisplatin. Patients No 7, 8, 15, 17, 20, 22, 26–31, 34 and 35 in the Table were treated with this protocol. The next protocol included methotrexate-bleomycin-cisplatin (MBC) and patients Nos 3, 9, 13, 19, 21 and 25 were treated with that protocol. Frequency: recycle every 3–4 weeks. Out of 40 patients 14 had recurrent tumors (Nos 7, 9, 14, 15, 18, 22, 25, 26, 28, 32–34, 36 and 39). The rest had primary tumors.

Serum samples from each patient were collected before, several times during and at the end of therapy (Table). Five to 10 ml of blood were collected in tubes without anticoagulants. After coagulation the blood was centrifuged and the serum obtained was stored at −20°C. Under these conditions, SADA is stable for more than 6 months.

For the biochemical detection of SADA a spectrophotometric technique (9) was adapted (5). The amount of oligonucleotides liberated during incubation was measured at 260 nm against a blank with addition of EDTA which blocks enzyme activity. The intraassay and interassay coefficients of variation were 3.6% and 8.5% respectively. The results are expressed in international kilo units/liter (kU/l).

Accepted for publication 21 January 1989.

Table

Pretreatment and posttreatment SADA values in 40 patients with head and neck cancer compared to their clinical response to therapy

Pat No.	Localization	Stage[a]	SADA (kU/l) before therapy	Therapy[b]	Phase I		Phase II			
					Samples after the beginning of therapy (days)	SADA (kU/l)	Clinical response[c]	After treatment (months)	SADA (kU/l)	Duration of response[d] (months)
1	Larynx	II	14	R	9	7	CR	3	27	10*
2	Tonsil	III	24	R	7	7	CR	3	34	9*
3	Epiglottis	III	10	R+C	10	5	CR	2	15	12*
4	Tonsil	III	6	R	15	3	CR	4	21	10*
5	Oropharynx	III	15	R	7	9	CR	3	27	8*
6	Lip	III	10	R	6	6	PR	2	8	5*
7	Epiglottis	III	10	R+C	15	4	PR	3	7	4*
8	Tongue	II	6	R+C	4	3	PR	2	5	4*
9	Buccal mucosa	III	28	R+C	7	11	PR	3	13	9
10	Nasal	III	34	R	7	10	PR	4	14	12*
11	Lip	II	42	R	7	11	PR	2	29	12*
12	Tonsil	II	12	R	8	8	PR	3	11	4*
13	Oropharynx	III	23	R+C	10	14	PR	4	19	15*
14	Maxillary gland	III	14	R+C	7	5	PR	3	11	7
15	Maxillary sinus	IV	9	R+C	8	1	PR	2	8	12
16	Parotid gland	II	4	R	5	2	PR	2	3	5*
17	Tonsil	IV	20	R+C	15	11	PR	3	15	10*
18	Gingiva	IV	8	R	5	4	PR	2	8	3*
19	Tonsil	IV	5	C	15	2	PR	4	4	6
20	Oropharynx	III	5	R+C	7	1	PR	2	4	6*
21	Buccal mucosa	IV	7	R+C	6	2	MR	2	2	4
22	Tonsil	III	9	R+C	10	6	MR	2	5	4
23	Tonsil	IV	8	R+C	7	5	MR	1	4	1
24	Buccal mucosa	III	8	R	7	6	MR	5	2	7
25	Tonsil	III	11	R+C	10	8	MR	4	3	8
26	Tongue	III	10	R+C	5	7	MR	5	4	9
27	Larynx	IV	27	R+C	7	4	MR	3	6	4
28	Maxillary sinus	IV	34	R+C	10	18	MR	4	11	9
29	Tongue	IV	32	R+C	15	17	MR	3	11	5
30	Buccal mucosa	IV	41	C	15	25	MR	2	4	3
31	Maxillary sinus	III	16	R+C	7	4	MR	4	4	6
32	Tongue	IV	9	R	7	10	NR	2	8	2
33	Parotid gland	IV	8	R	15	7	NR	1	6	1
34	Epiglottis	III	4	R+C	7	4	NR	2	3	2
35	Epiglottis	III	5	R+C	7	4	NR	3	4	3
36	Larynx	IV	5	R	15	5	NR	3	4	3
37	Nasal	IV	7	R	15	6	NR	1	4	1
38	Oropharynx	IV	7	R	15	6	NR	1	3	1
39	Parotid gland	IV	4	R	15	4	NR	4	2	6
40	Epiglottis	IV	3	R	15	4	NR	5	2	5

[a] TNM system and subclassification into four stages of disease: Stage I: T1 N0 M0; Stage II: T2 N0 M0; Stage III: T3 N0 M0; T1, T2, T3; N1, M0; Stage IV: T4, N0 or N1, M0; Any T, N2 or N3, M0; Any T, N1 or M1.
[b] R = radiotherapy; C = chemotherapy.
[c] Response to therapy. CR = complete response; PR = partial response; MR = minor response; NR = no response.
[d] Duration of response in months after the beginning of therapy. * indicates patients who are still alive up to the censored time.

Clinical information concerning diagnosis, treatment, and tumor response was collected during and at the end of therapy. Complete response (CR) was defined as the complete disappearance of all clinically detectable disease, partial response (PR) as a greater than 50% reduction in tumor size, and a minor response (MR) as a less than 50% reduction in tumor size. Non-response (NR) was defined as no regression in tumor size.

Statistical analysis was carried out using a one-way and a two-way analysis of variance (ANOVA), and the Scheffe test. A p value less than 0.05 was considered significant.

Results

Before treatment the level of SADA varied considerably from patient to patient (Fig. 1), as was also observed

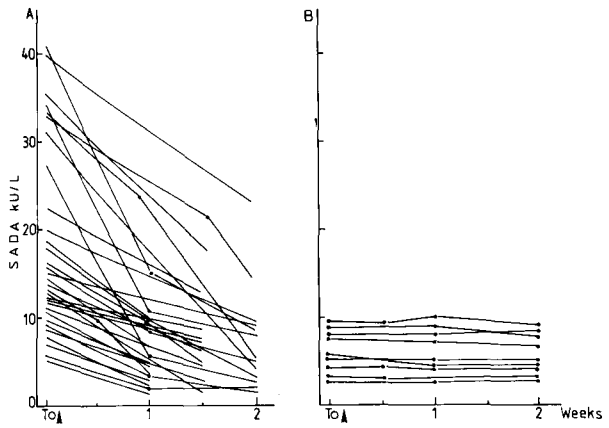

Fig. 1. SADA levels measured before (T0), during, or after the first treatment in 40 patients with head and neck cancer. A) 31 patients with initial response and B) 9 patients with no response. The arrow indicates the beginning at therapy.

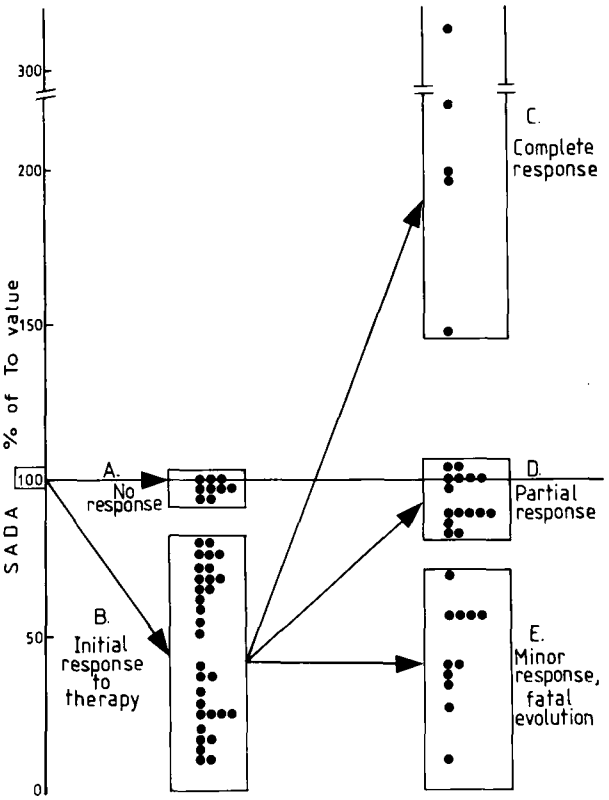

Fig. 2. Relative variations in SADA level in all the 40 patients analyzed according to their initial and final clinical response. The pretreatment value (T0) in each patient is considered as 100%.

in our previous clinical studies on other malignancies (5–8). Each patient was therefore individually analyzed and the SADA variations compared to the initial value determined before treatment.

Within two weeks from the beginning of the treatment 31 of the 40 patients had a primary clinical response while the remaining 9 patients showed no response.

A decrease in SADA was observed in all 31 responders between one to two weeks from the beginning of the first treatment (Fig. 1 A). No similar SADA variations were seen in the 9 non-responding patients (Figs 1 B, 2 A). The 2-way analysis of variance showed that there was a significant difference between the two groups ($p<0.01$), and also the Scheffe test indicated a significant difference.

Among the 31 responding patients who had an initial decrease in SADA during their first treatment (Fig. 2 B), three different groups could be identified according to their clinical response after continued therapy. Five patients with complete response showed a secondary increase in SADA largely exceeding the initial values (Fig. 2 C). All are still alive. A partial decrease in tumor size occurred in 15 patients and was associated with a less pronounced secondary increase of the SADA level which did not exceed the initial values (Fig. 2 D). In the third group, the 11 patients who showed minor clinical response and an initial decrease in SADA during the first treatment had no secondary increase of enzyme activity (Fig. 2 E); all 11 showed progressing tumor and died during the period of observation. One-way analysis of variance showed a significant difference between CR, PR and MR patients ($p<0.01$). The Scheffe test also showed a significant difference between these groups.

The Table gives details of the 40 patients analyzed.

Discussion

The SADA variations observed in this study can, at present, only be explained hypothetically. It has been shown that malignant tumors have characteristic changes in alkaline DNase activity which may depend on different factors such as natural inhibitor(s) (2, 9, 10–12). As yet the nature of the inhibitor(s) remains unknown. However, there are some indications that one inhibitor may be actin. Other mechanisms such as cell membrane permeability, non-specific inhibitory factors, or activators may also be involved (13–15).

Two hypothesis may explain the initial decrease in SADA after effective treatment: either an inhibitor is released into the circulation, or there might be an 'uptake' of the serum enzyme by the tumor. This event might be facilitated by the membrane alterations in necrotic cells after efficient therapy.

The secondary increase of SADA in patients with complete remission may imply a lack in enzyme uptake and/or a decrease in the quantity of the enzyme inhibitor(s) due to the disappearance of the tumor. If this hypothesis is correct it seems likely that the SADA values reached in complete remission represent the level present before the development of the tumor. Recent results in experimental animals support this hypothesis (16).

Similar SADA variations observed during treatment of malignant lymphomas (6), lung cancer (7), acute non-lymphoblastic leukemias (8), and in head and neck cancer, suggest that the behavior of alkaline DNase is not limited

to a specific kind of tumor. The results furthermore suggest that monitoring of SADA in patients with head and neck cancer could be a valuable means for early prediction of the efficacy of anticancer treatment. Additional clinical studies are, however, needed as prospective studies on larger series of patients including analyses of the correlation between SADA variations and survival data.

ACKNOWLEDGEMENTS

The authors thank Dr Bauduin, Dr G. Petit and Dr G. Delzenne, for providing all the clinical facilities and Ms V. Allaeys, Ms C. Heusdains and Ms P. S. Dolatabadi for their excellent technical assistance.

Request for reprints: Dr Anna Economidou-Karaoglou, Unité de Biochimie Toxicologique et Cancérologique, UCL 7369, B-1200 Bruxelles, Belgium.

REFERENCES

1. Brody S. Deoxyribonuclease activity and deoxyribonucleic acid synthesis in normal, regenerating precancerous and cancerous rat liver. Nature 1958; 182: 1386–7.
2. Daoust R, Amano H. Ribonuclease and deoxyribonuclease in experimental and human tumors by the histochemical substrate film method. Cancer Res 1963; 23: 131–4.
3. Taper HS, Brucher JM, Fort L. Activity of alkaline and acid nucleases in tumors of the human central nervous system. Cancer 1971; 28: 482–90.
4. Taper HS, Deckers C, Deckers-Passau L. Increase in nuclease activity as a possible means for detecting tumor cell sensitivity to anticancer agents. Cancer 1981; 47: 523–9.
5. Taper HS, Lans M, Economidou-Karaoglou A, de Gerlache J, Roberfroid M. Variations in serum alkaline DNase activity: A possible clinical test for therapeutic prognosis of human tumors. Anticancer Res 1986; 6: 949–56.
6. Economidou-Karaoglou A, Lans M, Taper HS, Michaux JL, Roberfroid M. Variations in serum alkaline DNase activity; A new means for therapeutic monitoring of malignant lymphomas. Cancer 1988; 61: 1838–43.
7. Economidou-Karaoglou A, Opsomer M, Petit G, Lans M, Taper HS, Roberfroid M. Characteristic variations of serum alkaline DNase activity in relation to tumor response to therapy and tumor prognosis in human lung cancer. Eur J Cancer Clin Oncol 1988; 24: 1337–43.
8. Economidou-Karaoglou A, Lans M, Taper HS, Michaux JL, Roberfroid M. Variations in serum alkaline DNase activity; A new means to assess early detection of relapse in patients treated for acute non-lymphoblastic leukemia. Blood 1989; (In press.)
9. Loiselle JM, Carrier R. Désoxyribonucléase sérique chez les cancéreux. Rev Cancer Biol 1963; 22: 341–6.
10. Malicka-Blaszkiewicz M, Roth JS. Evidence for the presence of DNase-actin complex in L1210 leukemia cells. Febs Letters 1983; 153: 235–9.
11. Mély-Goubert B, Matzku S, Bellgrau D. DNase I inhibitions in tumors of different metastasizing capacities: a possible index of invasiveness. Cancer Biochem Biophys 1983; 7: 39–44.
12. Taper HS. Reversibility of acid and alkaline deoxyribonuclease deficiency in malignant tumor cells. J Histochem Cytochem 1981; 29: 1053–60.
13. Dabrowska W, Cooper EJ, Laskowski M. A specific inhibitor for deoxyribonuclease. J Biol Chem 1949; 177: 991–2.
14. Lindberg U. Purification from calf spleen of two inhibitors of deoxyribunoclease I. Physical and chemical characterization of the inhibitor. Biochemistry 1967; 6: 323–35.
15. Lesca P. Sur les fonctions biologiques et la régulation de l'activité des désoxyribonucléases. Rev Eur Etud Clin Biol 1971; 16: 117–23.
16. Economidou-Karaoglou A, Brasseur F, Taper HS, Lans M, Roberfroid M. Typical variations in serum alkaline DNase activity in rats during growth and treatment of tumors sensitive or resistant to therapy. Int J Cancer 1989; 43: 956–9.