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Head Activator as a Potential Serum Marker for Brain Tumour Analysis

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Some human brain tumours, especially those derived from neuroectodermal or neural crest cells, contain elevated levels of head activator (HA). Such tumours release HA into the general circulation resulting in up to 1000-fold higher HA serum concentrations. The diagnostic value for brain tumour analysis was studied by measuring HA serum levels before and after human brain tumour surgery. It was found that complete removal of the tumour resulted in normal HA serum levels postsurgically. Incomplete resection was accompanied by a marked decrease, but not a return to normal HA levels. Surgery as such did not influence HA serum levels, since no change was observed pre- and postsurgically in patients with normal HA serum levels. This confirms the potential usefulness of HA as a serum marker to monitor brain tumour progression, successful tumour removal and relapse.
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INTRODUCTION

IN NEURO-ONCOLOGY only a few tumour markers are known which can be used for clinical diagnosis. With regard to specificity and sensitivity all known markers are not satisfactory. An ideal tumour marker should be present already at the subclinical stage of the disease, and it should be easily traceable, preferentially outside the brain, e.g. in the general circulation. This would allow to monitor the effects of surgery and therapy, and would help to diagnose tumour relapses.

Head activator (HA), a neuropeptide consisting of 11 amino-acids and a molecular weight of 1124 daltons, was originally isolated from the freshwater coelenterate hydra where it acts as a head-specific growth and differentiation factor [1]. Later a peptide of identical sequence was discovered in mammalian hypothalamus and intestine [2, 3]. Very high concentrations of HA were found in human tumours of the brain, in human gastrointestinal tumours, and others containing cells of neuroectodermal or neural crest origin [4]. Coincident with elevated levels of HA in tumour tissue, an increased systemic secretion of HA into the serum of tumour patients was observed [4]. The occurrence of HA early in brain development [5, 6], in brain tumours, and in cell lines derived from neural and neuroendocrine tumours hinted at a possible role of HA as a growth-regulating agent in mammalian brain development. This was supported by the finding that HA acts on such cell lines as an autocrine growth factor stimulating the progression from the G2 phase into mitosis [7]. In this paper we investigate the diagnostic value of HA as serum marker to monitor brain tumour occurrence and efficiency of brain tumour resection.

MATERIALS AND METHODS

Head activators

Synthetic head activator was from Bachem (Switzerland), Tyr^{11}-HA was synthesised [8] and radioiodinated by the chloramine-T method.

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Head activator radioimmunoassay (RIA)

The concentration of HA was measured with RIA using the polyclonal rabbit antiserum 53/13 at a dilution of 1:70000. Monomeric [^{125}I-Tyr^{11}]-HA (5000 cpm per tube) served as tracer [8, 9], synthetic (dimeric) HA was used for standardisation. Antiserum 53/13 yielded two times lower values than those previously obtained with antiserum 12/5 [4, 10], although both antibodies had been raised against the same antigen, namely HA coupled at the carboxy end to keyhole limpet haemocyanin by carbodiimide [10]. The difference in immunoreactivities is probably due to the fact that the two antisera contain different populations of antibodies interacting with one or the other HA conformation. A correction factor of 2 was introduced for antiserum 53/13 to obtain data comparable with those obtained with the antiserum 12/5. In the RIA as buffer 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) was used. BSA was extracted with methanol prior to use to remove traces of HA contamination. Samples containing HA were dissolved in 500 µl of this buffer and incubated overnight at 4°C with the antibody and the tracer. To construct standard curves 3, 10, 30 and 100 fmol of synthetic HA were used. Free and bound HA were separated by adding 200 µl of 1% bovine gamma-globulin in PBS and 1.5 ml of 20% polyethyleneglycol in 0.03% Triton X-100 in PBS. After thorough vortexing the bound HA was precipitated by centrifugation at 2000 *g* at 4°C for 30 min. The sediments were counted for their content of radioactivity in a gamma-counter. The assay was sensitive in the range of 3–100 fmol of HA [8].

Determination of HA concentration in the blood

Fresh blood samples were collected in 10 mmol/l EDTA to prevent degradation of HA by the angiotensin converting enzyme [11] and centrifuged at 700 *g* for 10 min. HA was extracted from 1 ml of the serum by adding 10 ml of methanol. After vortexing $(NH_4)_2SO_4$ was added to a final concentration of 0.03 mol/l followed by a centrifugation at 1000 *g* for 10 min. The sediment was reextracted with 5 ml of methanol at 60 °C for 30 min. The combined supernatants, after evaporation, were dissolved in 5 ml of water and absorbed to Seppak C-18 cartridges. HA was eluted from the cartridges with 20 ml of 80% methanol, after washing with 10 ml of water and 10 ml of

Table 1. Effect of intracranial surgery on HA serum levels

Source of serum	No. of cases	HA concentration (fm/ml)
Fasted healthy volunteers	16	67 (41)
Non-tumour neuroclinical patients	47	94 (87)
Non-tumour patients:		
Before neurosurgery		79 (45)
After neurosurgery	16	82 (48)

Mean (S.D.).

20% methanol. The eluted samples were evaporated and assayed in the RIA as described previously.

RESULTS

Head activator is present in human blood under normal conditions in concentrations of up to 200 pmol/l. Such values were measured in the sera of healthy fasted volunteers and of non-tumour neurosurgery patients [4]. No difference was found in HA levels before or after surgery in patients with initially low HA levels (Table 1). To investigate whether in patients with initially high HA serum levels HA concentration is correlated with tumour occurrence, HA content was determined before and after surgery. The diagnoses included benign and malignant brain tumours (Table 2).

In the sera of patients with meningioma (Fig. 1) the concentration of HA was clearly elevated before resection of the tumour. In single cases the HA concentrations were more than 50 times higher than the upper limit of the normal level. After surgery in 5 of 7 cases the HA concentrations dropped to a normal level

Table 2. Patients and diagnoses

Case	Sex, age	Diagnosis
1	M, 65	Glioblastoma
2	F, 69	Glioblastoma
3	M, 56	Glioblastoma
4	F, 71	Astrocytoma grade III
5	M, 46	Astrocytoma grade III
6	M, 29	Glioblastoma
7	M, 51	Glioblastoma
8	F, 52	Meningioma
9	F, 58	Meningioma
10	M, 46	Meningioma
11	F, 50	Malignant maningioma
12	M, 55	Meningioma
13	M, 48	Malignant meningioma
14	F, 52	Meningioma
15	M, 63	Small cell lung carcinoma, metastasis
16	M, 62	Lung adeno-carcinoma, metastasis
17	M, 67	Lung adeno-carcinoma, metastasis
18	F, 77	Mamma carcinoma, metastasis
19	M, 63	Kidney carcinoma, metastasis
20	F, 65	Pituitary adenoma, eosinophilic
21	M, 60	Pituitary adenoma, chromophobic
22	F, 49	Pituitary adenoma, eosinophilic
23	M, 62	Pituitary adenoma, eosinophilic
24	F, 34	Medulloblastoma
25	M, 30	Acusticus neurinoma
26	M, 14	Cerebral angioma

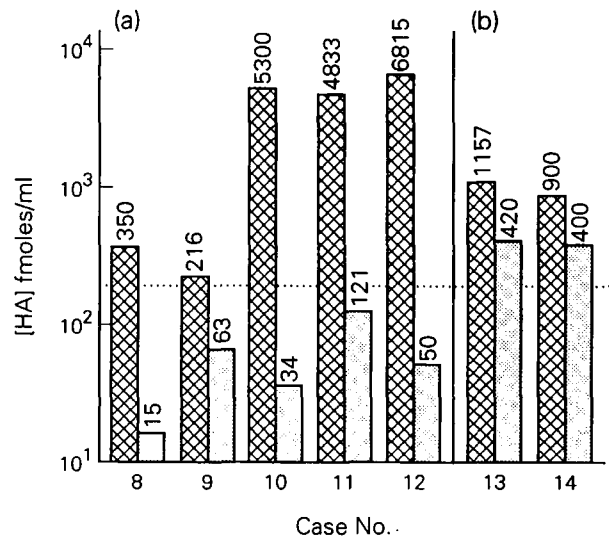

Fig. 1. Levels of HA in sera of neurosurgery patients with meningeoma before (hatched bars) and after (grey bars) surgery of the tumour. The broken line indicates the normal upper limit of HA concentrations in sera of neuroclinical patients without tumour diagnosis. (a) HA level in serum after operation lower than normal upper limit. (b) HA level in serum after operation higher than normal upper limit.

(cases 8–12). In these cases a total surgical resection of the tumours had been achieved. In the other two cases (cases 13, 14) the HA levels decreased significantly to less than half of the value measured before surgery. As explanation for the still increased HA serum levels after surgery we observed that in case 13 the tumour was malignant and could not be removed completely, and that in case 14 the tumour had invaded the base of the skull. In both cases a total extirpation of the tumour was not possible. Consequently we suggest that the elevated HA levels after surgery indicated presence of remaining tumour tissue.

As expected this was in particular true for invasively growing glioblastoma, which very often cannot be removed totally by surgical methods. It is evident from Fig. 2 that the reduction of the tumour mass led in all cases to a significant decrease in HA serum level. In the sera of two patients (cases 1 and 2) the HA content decreased to a tenth of the value before surgery. For 2 cases out of 7 a reduction of the HA concentration to the normal level was observed after surgery (cases 5 and 7).

In Fig. 3 we show the results for patients with metastatic brain tumours. In the serum of a patient with a metastasis of a small cell lung carcinoma (case 15) we found a 1000-fold increase of HA concentration before surgery compared to normal HA levels. Postsurgically, a normal HA content was measured. As reported before, patients with small cell lung carcinoma and with metastases of such tumours to the brain show highly elevated HA levels [4]. In the sera of two of the metastatic tumour patients, one lung adeno-carcinoma and the other kidney carcinoma (cases 17 and 19), postsurgically still elevated HA levels were detected. This could indicate presence of additional metastases, not necessarily in the brain. The metastasis of the mamma carcinoma (case 18) was not accompanied by an elevated HA serum level. This correlates with earlier findings that mamma carcinoma do not produce additional HA (unpublished results). For all other patients with metastatic brain tumours a clear postsurgical decrease of seral HA concentration was measured.

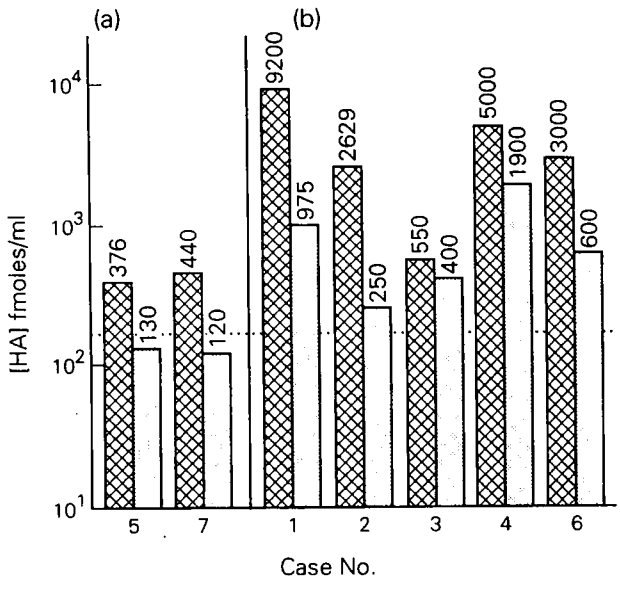

Fig. 2. Levels of HA in sera of neurosurgery patients with glioblastoma and astrocytoma before (hatched bars) and after (grey bars) surgery of the tumour. The broken line indicates the normal upper limit of HA concentrations in sera of neuroclinical patients without tumour diagnosis. (a) HA level in serum after operation lower than normal upper limit. (b) HA level in serum after operation higher than normal upper limit.

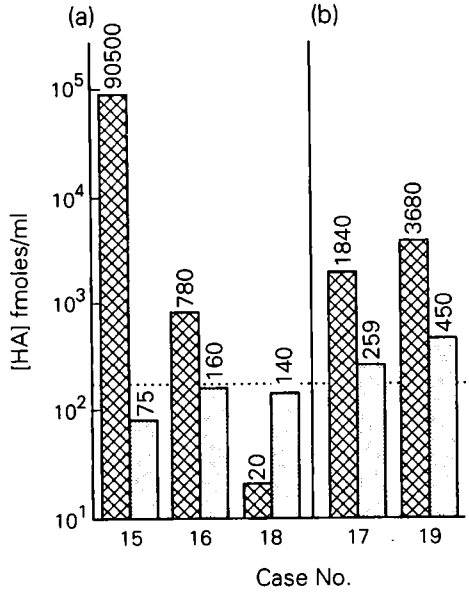

Fig. 3. Levels of HA in sera of neurosurgery patients with metastatic brain tumours before (hatched bars) and after (grey bars) surgery of the tumour. The broken line indicates the normal upper limit of HA concentrations in sera of neuroclinical patients without tumour diagnosis. (a) HA level in serum after operation lower than normal upper limit. (b) HA level in serum after operation higher than normal upper limit.

The various tumours (Fig. 4) included pituitary adenoma (cases 20, 21, 22, 23), medulloblastoma (case 24), acusticus neurinoma (case 25) and angioma (case 26). One of the pituitary tumours was a chromophobic adenoma (case 21). In this case a 60-fold increase in HA serum level was measured. The other three pituitary tumours with a less pronounced increase in seral

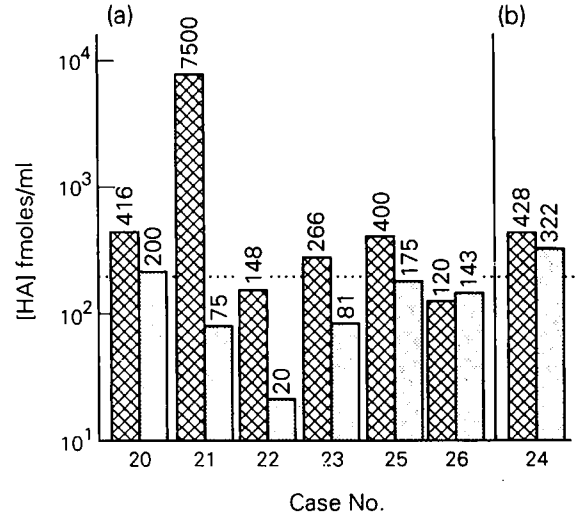

Fig. 4. Levels of HA in sera of neurosurgery patients with various brain tumours (see Table 1) before (hatched bars) and after (grey bars) surgery of the tumour. The broken line indicates the normal upper limit of HA concentrations in sera of neuroclinical patients without tumour diagnosis. (a) HA level in serum after operation lower than normal upper limit. (b) HA level in serum after operation higher than normal upper limit.

HA level were eosinophilic adenoma. Postsurgically in all cases a drop in HA serum level to normal values was observed. Only 1 patient (case 24) with a partial tumour extirpation still showed a slightly elevated HA serum level.

DISCUSSION

In the present paper we try to establish HA as an immunological serum marker for brain tumour analysis. We found that increased HA concentrations are detectable in the sera of patients with various types of brain tumours derived from cells of neuroectodermal or neural crest origin, such as, e.g. glioblastoma, astrocytoma, meningioma and metastasis of small cell lung carcinoma. These HA serum levels decrease significantly after tumour removal hinting at a correlation between tumour occurrence in the brain and HA concentration in the serum. After total tumour resection HA serum levels drop to normal values as observed for non-clinical volunteers and for neuroclinical patients without tumour diagnosis. HA serum concentrations drop significantly, but not to normal levels, if the tumour is only partially reduced by surgery. Since surgery as such does not influence HA serum concentrations in other non-tumour patients, we suggest that monitoring HA serum levels may help to evaluate the efficiency of brain tumour resection, to study brain tumour progression and to facilitate the early detection of tumour relapses.

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Long-term Prognostic Significance of Thymidine Labelling Index in Primary Breast Cancer

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Tumour growth rates, as measured by incorporation of tritiated thymidine, have been reported as being of prognostic importance in breast cancer. We have measured the thymidine labelling index (TLI) of 185 early breast cancers, followed-up for a minimum of 8 years. Above median TLI was associated with higher tumour grade, but not with other prognostic factors. TLI was not predictive of survival in either univariate or multivariate analysis. The inter- and intra-observer reproducibilities of TLI measurements were poor, which may be a factor limiting its usefulness as a prognostic indicator in breast cancer.
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INTRODUCTION

THERE HAVE been a number of publications evaluating the usefulness of thymidine labelling in breast cancer since it was first applied by Johnson and Bond [1]. However, little attention has been paid to the reproducibility of the measurement itself. We present here a cohort of patients whose primary treatment was by surgery alone, followed up for a minimum of 8 years, in whom thymidine labelling index (TLI) of the primary tumour was measured at initial presentation. We also report the inter- and intra-observation variability in the assessment of TLI.

PATIENTS AND METHODS

Patients

185 tumours were studied between 1978 and 1982. All had stage I and II breast cancer, treated by mastectomy and axillary dissection. No patients were given adjuvant therapy. They have been prospectively followed up, and flagged in the Regional Cancer Registry to ensure accuracy of mortality data. The close of the study was taken as 1 January 1990, when the minimum follow-up of surviving patients was 93 months.

Methods

(*a*) *Thymidine labelling.* The TLI was measured by the method of Meyer and Bauer [2]. Fresh tumour was divided into five 2 mm cubes, which were added to tubes containing 5 ml of RPMI and 0.2 ml of ^{3}H-thymidine (925 μBq/ml; specific activity 1.6 Tbq/mmol). Tubes were incubated in a shaking water bath for 2 h at 37°C, at 3 atm produced by addition of 10 ml of a 95% O_2/5% CO_2 mixture. Tissue was then fixed in formalin and wax embedded. Five micron sections were mounted on histological slides and Kodak AR stripping film applied. Autoradiographs were exposed at 4°C for 28 days, and counterstained with haematoxylin and eosin.

Labelling was assessed in 2000 nuclei per tumour, four separate areas of 100 nuclei on each of five slides. Nuclei were considered to be positive if there were more than 10 reduced silver grains over them, although negative nuclei never demonstrated more than three grains. The TLI was taken as the proportion of positive nuclei. Inter- and intra-observer variability were assessed in 20 tumours.

(*b*) *Other data.* Histological grade was assigned by a single pathologist according to the criteria of Bloom and Richardson. ER assays were performed using the dextran coated charcoal

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