Serum hyaluronic acid in patients with disseminated neoplasm

G MANLEY, CHRISTINE WARREN

From the Department of Chemical Pathology, Torbay Hospital, Torquay, Devon

SUMMARY Hyaluronic acid concentrations were measured by a laser nephelometric assay in serum samples from 50 patients with advanced disseminated neoplasm and 50 healthy controls matched for age and sex. The identity of hyaluronic acid was confirmed by a combination of electrophoretic and enzymatic techniques. The mean serum hyaluronic acid concentration for the control group was 1.09 mg/l, with a range of 0–4 mg/l. The mean concentration for patients with neoplastic disease was 10.38 mg/l, with a range of 0–100 mg/l. Sixty two per cent of the patients with disseminated neoplasm had serum hyaluronic acid concentrations above the control range. There was no correlation between the increased concentration of hyaluronic acid and tumour type, serum bilirubin, serum alkaline phosphatase, or serum urea concentrations. There was a higher incidence of hypercalcaemia in patients with increased hyaluronic acid concentrations, but the correlation between hyaluronic acid and calcium concentrations was not significant. In view of the possible role of hyaluronic acid in cellular differentiation and morphogenesis the finding of increased hyaluronic acid concentrations in patients with advanced neoplastic disease may be of fundamental importance in cancer biology.

Hyaluronic acid is a high molecular weight glycosaminoglycan composed of alternate glucosamine and glucuronic acid units. It has a key structural role in connective tissue ground substance¹ and is also found in unusually high concentrations in fetal tissues.² Since 1957 there have been sporadic reports of hyaluronic acid production by certain tumours.^{3–5} Hyaluronic acid is present in the urine of neonates but is not normally found in the urine of adults.⁶ In 1978 we reported the presence of hyaluronic acid in the urine of about 30% of patients with disseminated neoplasm.⁷ Having developed a laser nephelometric assay for serum glycosaminoglycans we subsequently found that hyaluronic acid may be detected in low concentration in the serum of some healthy individuals.⁸ We now report the presence of a striking increase in serum hyaluronic acid concentration in some patients with disseminated neoplasm.

Patients and methods

Fifty patients were studied, all of whom had well documented evidence of advanced neoplastic disease, including hepatic or skeletal metastases, or both. Samples of serum from venous blood were paired

with samples from 50 healthy volunteers matched for age and sex and stored at –20°C for up to four weeks before analysis.

Hyaluronic acid assays were performed by the method of Warren and Manley,⁸ which entails addition of cetylpyridinium chloride to serum and measurement of the cetylpyridinium-chloride-polyanion complexes by laser nephelometry before and after digestion with streptomyces hyaluronidase. For qualitative study of serum glycosaminoglycans serum samples (5 ml) were digested with papain and dialysed against water. Glycosaminoglycans were isolated by precipitation with cetylpyridinium chloride; the complexes were dissociated in 2 M sodium chloride and finally precipitated as sodium salts in ethanol. The isolated glycosaminoglycans were separated by electrophoresis on cellulose acetate membrane in veronal acetate buffer, pH 9.2, and calcium acetate buffer, pH 5.0, and stained with 1% alcian blue in 2% acetic acid.⁹ Hyaluronic acid, heparan sulphate, chondroitin sulphate, and streptomyces hyaluronidase were obtained from Miles laboratories. Serum hyaluronic acid was identified by its electrophoretic mobility, which was identical with that of a standard sample of hyaluronic acid at both pH values, and its total destruction when incubated with streptomyces hyaluronidase (15 turbidity reducing units (TRU)/mg glycosaminoglycan) for two hours at 37°C.

Serum concentrations of sodium, potassium, chloride, bicarbonate, urea, total protein, albumin, bilirubin, alkaline phosphatase, calcium, and phosphate were measured by continuous flow analysis using a Vickers SP120 RT analyser. Calcium values were corrected to an albumin value of 40 g/l, assuming 0.025 mmol calcium/g albumin.

Results

Serum hyaluronic acid concentrations in the control group were in the range <0.5 – 4 mg/l, with a mean of 1.09 mg/l; this is in close accord with normal values previously found with this method.⁸ Serum hyaluronic acid concentrations in the patients with neoplastic disease were in the range <0.5 – 100 mg/l, with a mean of 10.38 mg/l. Sixty eight per cent of these patients had values above 4 mg/l, and in 48% this was above 5 mg/l (fig 1).

Because the precise upper limit of normal for serum hyaluronic acid concentration has not yet been established, the patients with neoplastic disease were divided into three groups: group 1 consisted of patients whose serum hyaluronic acid concentration was clearly increased—that is, >5 mg/l (24 patients); group 2 was an intermediate group with serum hyaluronic acid concentrations of 4 – 5 mg/l (14 patients);

and group 3 consisted of patients whose serum hyaluronic acid concentration was clearly normal—that is, <4 mg/l (12 patients). In group 1 83% of patients had increased serum concentrations of alkaline phosphatase, 46% had increased calcium values, 46% had increased urea values, and 36% had increased bilirubin values (table). In group 2 the results were as follows: increased alkaline phosphatase 93%, increased calcium 29%, increased urea 50%, and increased bilirubin 36%. In group 3 the figures were increased alkaline phosphatase 83%, increased calcium 25%, increased urea 33%, and increased bilirubin 33%. There was no clear correlation between serum hyaluronic acid concentrations and tumour type.

Glycosaminoglycans isolated from serum samples with raised hyaluronic acid concentrations by laser nephelometry showed an alcian blue positive electrophoretic band of mobility identical with that of standard hyaluronic acid at pH 9.2 (fig 2) and pH 5.0. This band was totally destroyed by incubation with streptomyces hyaluronidase, which is highly specific for hyaluronic acid.¹⁰ This band could not be clearly identified in control samples or in samples from patients with neoplastic disease with normal serum hyaluronic acid concentrations.

Discussion

The finding of increased, in some cases greatly increased, serum concentrations of hyaluronic acid in more than half of the patients with disseminated neoplasm was unexpected. Clearly, the trace of hyaluronic acid found in the urine of some patients with disseminated neoplasm was but a shadow of the striking phenomenon reported here.⁷

The increased serum concentrations of hyaluronic acid in patients with neoplastic disease are unlikely to be due to hepatic or renal failure. There was no significant difference in serum bilirubin, alkaline phosphatase, or urea concentrations between the patients with increased serum hyaluronic acid concentrations and those with normal values. Slight increases in serum hyaluronic acid concentration have been reported in some patients with inflammatory disease, including rheumatoid arthritis, (up to 7.0 mg/l), but the mean value for this group (1.07 mg/l) was not significantly different from that for the control group.¹¹ The incidence of hypercalcaemia in the patients with raised serum hyaluronic acid concentrations (46%) was higher than that in patients with normal serum hyaluronic acid values (25%), but this difference was not significant ($p > 0.1$). We have not encountered similar increases in serum hyaluronic acid concentration in cases of non-neoplastic hypercalcaemia.¹¹ The series reported here

Fig 1 Serum hyaluronic acid concentrations in 50 patients with disseminated neoplasm and in 50 controls matched for age and sex.

Clinical and biochemical data* on 24 patients with disseminated neoplasm and raised serum hyaluronic acid concentrations ($> 5 \text{ mg/l}$)

Case No	Age (years)	Sex	Tumour type	Serum hyaluronic acid (mg/l)		Serum alkaline phosphatase (U/l) (20-90 U/l)	Serum calcium (corrected)† (mmol/l) (2.1-2.6 mmol/l)	Serum urea (mmol/l) (2.5-6.6 mmol/l)	Serum bilirubin ($\mu\text{mol/l}$) (5-17 $\mu\text{mol/l}$)
				Patient	Control				
1	77	F	Adenocarcinoma of stomach	100.0	2.2	> 500	3.23	8.9	87
2	73	F	Squamous carcinoma of bronchus	7.0	1.0	182	3.29	4.0	6
3	27	F	Adenocarcinoma of ovary	7.0	< 0.5	80	2.31	3.5	8
4	76	M	Adenocarcinoma of colon	7.0	0.5	298	2.78	23.0	11
5	65	M	Adenocarcinoma of pancreas	7.0	< 0.5	305	2.14	2.8	7
6	84	M	IgG myeloma	10.0	2.0	197	2.42	3.7	12
7	58	F	IgG myeloma	8.0	0.75	70	2.39	4.2	10
8	66	M	IgG myeloma	10.0	< 0.5	85	2.50	9.0	8
9	86	F	Adenocarcinoma of colon	6.0	0.5	354	2.41	8.2	9
10	74	F	Squamous carcinoma of bronchus	13.5	1.0	132	3.03	6.0	5
11	84	F	Adenocarcinoma of breast	6.0	2.0	133	2.32	5.0	10
12	64	M	Adenocarcinoma of stomach	30.0	< 0.5	> 500	2.72	5.0	28
13	40	F	Adenocarcinoma of breast	52.5	0.75	464	2.74	8.9	72
14	71	F	Adenocarcinoma of breast	8.0	2.5	412	2.25	4.0	8
15	67	F	Adenocarcinoma of ovary	64.5	1.0	> 500	2.75	4.5	15
16	61	M	Squamous carcinoma of bronchus	24.0	0.5	> 500	2.85	9.9	160
17	53	F	Adenocarcinoma of ovary	22.0	3.0	> 500	3.81	8.5	7
18	55	F	Adenocarcinoma of colon	10.0	0.75	126	2.46	3.0	6
19	84	F	Adenocarcinoma of colon	12.5	1.0	> 500	2.44	6.3	27
20	67	F	Undifferentiated carcinoma of breast	8.0	2.2	> 500	2.12	9.7	> 300
21	74	F	Squamous carcinoma of bronchus	8.0	0.5	73	3.79	17.0	14
22	48	F	Adenocarcinoma of breast	7.0	1.5	> 500	2.44	5.0	66
23	64	M	Adenocarcinoma of colon	6.0	< 0.5	> 500	2.52	5.4	53
24	65	F	Undifferentiated carcinoma of breast	7.0	2.5	223	3.26	5.9	5

*Normal ranges are given in parentheses.

†Calcium values were corrected to an albumin of 40 g/l, assuming 0.025 mmol calcium/g of albumin.

Fig 2 Electrophoretic patterns of (a) standard glycosaminoglycan mixture containing chondroitin-4-sulphate (CS), heparan sulphate (HS), and hyaluronic acid (HA); (b) glycosaminoglycans isolated from serum of one patient (case 1) with disseminated neoplasm; and (c) glycosaminoglycans isolated from serum of healthy control matched for age and sex. Electrophoretic origin is indicated by 0. (Veronal acetate buffer, pH 9.2; alcian blue stain.)

Fig 3 Serial measurements of serum hyaluronic acid concentration in one patient with disseminated carcinoma of breast showing rapid rise towards point of death (case 13).

is too small to allow firm conclusions to be made about the relation between hypercalcaemia and hyaluronic acidemia in patients with neoplastic disease, but we suspect that both may be independent phenomena associated with terminal neoplastic disease. In our previous study the patients with hyaluronic acid in their urine were all dead at follow up after three months.⁷ In the present study we were able to assay serial samples in three patients and found rapidly rising serum hyaluronic acid concentrations up to the point of death (fig 3).

Possible reasons for the increased serum concentrations of hyaluronic acid in patients with neoplastic disease include a release of hyaluronic acid from connective tissue ground substance by invading neoplastic cells, the inhibition of hyaluronic acid catabolism by the production of hyaluronidase inhibitors, and the production of hyaluronic acid by the tumour cells themselves or as a result of their interaction with normal cells.

Increased amounts of hyaluronic acid have been shown in some human tumour tissues—notably, neuroblastoma, breast tumours, adenocarcinoma of colon, mesothelioma, and Wilms' tumour.⁴ Increased serum hyaluronic acid concentrations have been reported in patients with neuroblastoma³ and in a case of Wilms' tumour.¹² In neither of these reports was the source of the hyaluronic acid clearly established, but in the case of Wilms' tumour the authors concluded that the production of hyaluronic acid was related to the tumour since concentrations decreased promptly and dramatically on treatment with vincristine and became normal after the tumour was removed. Further evidence that hyaluronic acid may be a product of tumour cells comes from the finding that transformation of green monkey kidney cells with SV₄₀ leads to an accumulation of excess hyaluronic acid as compared with their counterpart normal cells.¹³ On the other hand the work of Knudson *et al* suggests that the increased production of hyaluronic acid in patients with neoplastic disease may depend on an interaction between tumour cells and normal fibroblasts.¹⁴ They found that coculture of human LX-1 lung carcinoma cells and normal human fibroblasts produced three times more hyaluronic acid than the sum of that produced by LX-1 and fibroblast cultures grown separately.

As hyaluronic acid is found in particularly high concentrations in fetal tissues,² increased serum hyaluronic acid concentrations in patients with neoplastic disease may be an oncofetal phenomenon. It would be a particularly interesting one as hyaluronic acid, as a non-protein is not (in our current concept of genetic information transfer) thought to be a direct gene product.

Of central importance in cellular differentiation,

and hence in neoplasia, is the positional sense of a cell in relation to its fellows. Little is understood of mechanisms here, but the intricate patterns of the molecular scaffolding of the intercellular matrix may be important. In connective tissue ground substance hyaluronic acid exists as long intercellular chains of high molecular weight, into which are linked proteoglycans containing variable chains of chondroitin sulphates and keratan sulphates.¹

In the field of pure speculation, the detailed molecular organisation of the intercellular hyaluronate-proteoglycan complexes would seem morphologically suited to a role in intercellular information transfer. In even wilder speculation, might a cell surface defect in the neoplastic cell, which damages its ability to recognise the hyaluronic acid based intercellular scaffolding, lead to an increased production of hyaluronic acid analogous to the increased cellular synthesis of cholesterol in low density lipoprotein receptor defect?¹⁵ Of course, there is no evidence for this at present, but there is already some evidence that hyaluronic acid has a role in intercellular communication,¹⁶ cell adhesion,¹⁷ and morphogenesis.¹⁸ Against this background the finding of considerably raised serum hyaluronic acid concentrations in patients with a wide range of neoplastic diseases may point to a phenomenon of fundamental importance in neoplasia.

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Requests for reprints to: Dr G Manley, Department of Pathology, Torbay Hospital, Torquay, TQ2 7AA, England.