Serum beta-lipoprotein and other specific protein concentrations in patients with immunocytoma

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SYNOPSIS Serum β -lipoprotein and other specific protein concentrations were measured in 56 patients suffering from multiple myelomatosis, 'benign' paraproteinaemia or Waldenström's macroglobulinaemia and in 56 control subjects. The mean level of β -lipoprotein in untreated patients with multiple myelomatosis and macroglobulinaemia was significantly lower than that of the controls. Patients who responded to chemotherapy showed a rapid return to normal of the β -lipoprotein concentration, while the level remained unchanged in most of those who did not.

The majority of patients suffering from multiple myelomatosis and Waldenström's macroglobulinaemia show disturbances in serum and urine proteins (Hobbs, 1971). Less well established is the presence of a decreased β -lipoprotein (BLP) concentration (Burstein and Fine, 1959). The present investigation was undertaken to examine the relationship between 'benign' paraproteinaemia, multiple myelomatosis, and Waldenström's macroglobulinaemia and five specific protein fractions before and after therapy. The serum proteins measured were albumin, transferrin, BLP, α_1 -antitrypsin, and C₃.

Subjects

CONTROLS

Venous blood samples were taken from 56 blood donors who were matched with the patient group for sex and age.

PATIENTS

Blood and urine were obtained from 56 patients attending the Haematology Clinic of the Johannesburg General Hospital. Forty-six patients were suffering from multiple myelomatosis, seven patients had a serum paraprotein but no other evidence of myelomatosis, and three patients had clinical and laboratory features of Waldenström's macroglobulinaemia. In the myeloma group there were 32 patients with an IgG paraprotein, seven with IgA, one with IgM, one with IgD, and five with Bence-

Jones myelomatosis. In all patients, samples were obtained at the time of presentation, and repeatedly at intervals of 3-4 months over a period of up to 63 months.

Patients with myelomatosis were all treated with prednisolone and melphalan using the dosage regimen described by Alexanian *et al* (1968), patients with Waldenström's macroglobulinaemia received a daily dose of chlorambucil, and those who had only a paraprotein received no specific therapy. The patients were segregated into two groups according to their responsiveness to therapy as gauged by means of criteria based upon those suggested by the Chronic Leukaemia/Multiple Myeloma Task Force of the National Cancer Institute (Bernard *et al*, 1974).

Methods

SERUM IMMUNODIFFUSION STUDIES

Serum immunoglobulins G, A, and M, BLP, transferrin, α_1 -antitrypsin, and C₃ were estimated by radial immunodiffusion using a technique previously described (Shulman, 1973). The working standards used were prepared from a carefully calibrated blood donor serum pool stored in aliquots at -20°C . A freshly thawed pool aliquot was always used to prepare working standards. Repeated measurements of total BLP concentration in individual samples were carried out after storage at -20°C to assess the effect of freezing. Subsequent repeated measurements, after refreezing and thawing the same samples several times, were also made.

Group	Number	Age (years)	BLP (g/l)	Cholesterol (mmol/l)	Haemoglobin (g/dl)	Transferrin (g/l)	Albumin (g/l)	Para-protein (g/l)	α_2 -anti-trypsin (g/l)	C ₃ (g/l)
1 Blood donor controls	56	62 ± 9	4.5 ± 0.8	5.6 ± 0.7		2.7 ± 0.5	32 ± 3		2.2 ± 0.4	0.7 ± 0.1
2 Total immunocytoma group before therapy	49	62 ± 9	2.4 ± 1.3 P < 0.001	3.8 ± 1.3 P < 0.05		1.9 ± 0.7 P < 0.01	31 ± 6 NS	35 ± 16	2.6 ± 0.7 P < 0.05	0.8 ± 0.3 NS
3 'Benign' paraproteinaemia	7	61 ± 9	4.8 ± 0.8 NS	6.1 ± 0.8 NS		2.7 ± 0.6 NS	34 ± 5 NS	14 ± 4	2.3 ± 0.6 NS	0.9 ± 0.3 NS
4 Non-responders before therapy	30	62 ± 8	2.4 ± 1.4	3.7 ± 1.3	10.3 ± 2.3	1.8 ± 0.8	31 ± 6	35 ± 6	2.7 ± 0.7	0.8 ± 0.3
5 After therapy	12	61 ± 10	2.8 ± 1.3 NS	3.7 ± 1.3 P < 0.05	10.3 ± 2.6 NS	2.1 ± 0.6 NS	30 ± 5 NS	29 ± 12 NS	2.7 ± 0.7 NS	0.8 ± 0.2 NS
6 Responders before therapy	19	61 ± 9	2.3 ± 1.0	3.9 ± 1.2	12.4 ± 1.4	2.2 ± 0.6	32 ± 5	34 ± 18	2.4 ± 0.6	0.8 ± 0.2
7 After therapy	19	61 ± 9	4.1 ± 1.3 P < 0.001	5.7 ± 1.9 P < 0.001	12.7 ± 1.7 NS	2.3 ± 0.5 P < 0.05	33 ± 4 NS	18 ± 13 P < 0.001	2.7 ± 0.6 NS	0.8 ± 0.2 NS

Table Concentrations of BLP and other specific proteins, cholesterol, and haemoglobin in normal controls and patients with immunocytoma together with serum paraprotein levels in patients (excluding those with Bence-Jones myelomatosis).

NS = not significant

Patient results are divided into groups to indicate mean values ($\pm$ SD) at the time of presentation and after chemotherapy. P values in groups 2 and 3 are derived by using the Student's *t* test for non-paired data to compare the values in groups 1 with those in groups 2 and 3 respectively. P values in groups 5 and 7 are derived from Student's test for paired data, the comparison being made between values before and after therapy.

ELECTROPHORETIC STUDIES

Lipoprotein electrophoresis was carried out on fresh fasting serum on cellogel and quantitative protein electrophoresis on cellulose acetate (Beckman Microzone Apparatus). Urine was examined for the presence of Bence-Jones protein and the amount was measured by electrophoresis as previously described (Shulman *et al*, 1974). Immunochemical confirmation was performed with agar gel immunoelectrophoresis.

ULTRACENTRIFUGATION

Fresh fasting serum was ultracentrifuged for 16 hours at 105 000 *g* in a density gradient solution of 1.006 (Hatch and Lees, 1968). The proportion of BLP in the low density lipoprotein (LDL) and the very low density lipoprotein (VLDL) fractions was assayed by immunodiffusion.

Results

Evaluation of the technique for BLP estimation showed the method to be reliable. Precision was good (coefficient of variation of the mean of 52 replicates was 3.3 %). No significant alteration in concentration measured immunochemically in matched pairs of samples occurred as a result of freezing BLP apoprotein. Repeated testing after six months' storage at -20°C also showed no significant alteration in BLP concentration.

ULTRACENTRIFUGAL AND LIPOPROTEIN ELECTROPHORETIC STUDIES

In ultracentrifuged fasting samples from all the normal controls as well as from seven patients with

'benign' paraproteinaemia and 10 with multiple myeloma tested before administration of chemotherapy, the VLDL fraction accounted for up to 5 % of the total BLP. The lipoprotein electrophoretic patterns on cellogel were qualitatively normal in all controls and in the seven patients with 'benign' paraproteinaemia as well as in the 10 pretherapy myeloma patients.

CONTROLS

Results are shown in the table. There was a fairly close correlation between serum cholesterol and BLP concentrations among the controls ($r = 0.6537$, $P < 0.01$). There was no correlation between age and BLP ($r = 0.3026$, P NS).

PATIENTS AT PRESENTATION

The mean values for BLP and cholesterol were significantly lower (table) in 46 patients with multiple myeloma and the three with macroglobulinaemia at clinical presentation than they were in the normal controls. The correlation coefficient between cholesterol and BLP was 0.7373 ($P < 0.01$). The seven patients with 'benign' paraproteinaemia showed BLP and cholesterol levels similar to those of the controls (table) over a period of 60 months.

RESPONDERS AFTER THERAPY

Eighteen of the 46 patients with multiple myelomatosis and one of the three patients with Waldenström's macroglobulinaemia responded to therapy. Analyses were made when the period of follow-up, measured from the time of starting treatment, lay between 10 and 45 months (median 24.4 months). Their mean BLP and cholesterol levels were similar to those of the

normal controls at this stage (table); however, both fractions were significantly raised when compared with their initial measurements ($P < 0.001$). In many instances the rise in BLP preceded the fall in the rate of excretion of Bence-Jones protein, which in turn usually preceded the fall in the serum 'M' component. The fall in the mean serum paraprotein level in 18 patients after therapy was significant (table). The nineteenth patient was suffering from Bence-Jones myelomatosis. It is also interesting to note that in seven patients the serum cholesterol was between 3.9 and 6.0 mmol/l at the time of presentation. All of them became hypercholesterolaemic (serum/cholesterol > 6.5 mmol/l) while on therapy, one patient reaching a level of 10.0 mmol/l. The correlation between BLP and cholesterol in the whole group was maintained while on therapy ($r = 0.8872$, $P < 0.01$).

NON-RESPONDERS AFTER THERAPY

Thirty patients (28 with myeloma; two with Waldenström's macroglobulinaemia) did not respond to therapy and 15 died during their first hospital admission. BLP and cholesterol values in this group of patients were significantly lower than those of normal controls ($P < 0.001$). Analyses were repeated when the period of follow-up, measured from the time of starting treatment, lay between two and 37 months (median 10.7 months). The 15 surviving patients showed no consistent change in their BLP or cholesterol levels during the treatment period. Although there was a significant correlation between cholesterol concentration and BLP initially, this was lost after the patients had received therapy ($r = 0.4215$, P NS). Serum paraprotein mean levels showed no difference in this group of patients before and after chemotherapy (table). Of the 15 patients who were followed up, only five were still alive after 24 months.

RELATIONSHIP BETWEEN HYPOCHOLESTEROLAEMIA AND ANAEMIA

There was no significant correlation between BLP and haemoglobin concentration in our two patient groups at the time of presentation (responders $r = 0.4555$, P NS; non-responders $r = 0.0083$, P NS) or after therapy (responders $r = 0.3599$, P NS; non-responders $r = 0.2200$, P NS). In the analysis of the relationship between cholesterol and haemoglobin values the same pattern was found.

MEASUREMENTS OF OTHER SPECIFIC PROTEINS

Four other specific proteins, namely albumin, α_1 -antitrypsin, C_3 , and transferrin, were measured

(table). The group of patients with multiple myeloma or macroglobulinaemia had a mean transferrin level which was significantly lower and a mean α_1 -antitrypsin level which was significantly higher than the respective mean values in controls. C_3 levels were normal in the patient groups.

Discussion

The method of radial immunodiffusion used for estimating BLP appeared to be reliable. The BLP concentration measured immunochemically was not altered by freezing the specimen or storing it at -20°C in contrast to the situation seen on electrophoresis where there is a loss of electrophoretic sharpness after freezing had taken place.

Burstein and Fine (1959) first reported the presence of decreased BLP in patients with multiple myelomatosis and Waldenström's macroglobulinaemia. The results of the present investigation confirm their observations in untreated patients. Responsiveness to therapy was, however, accompanied by a rapid return of BLP towards normal. Patients who failed to show any biochemical or clinical improvement remained with persistently decreased BLP levels. In patients with 'benign' paraproteinaemia BLP was normal.

The reason for the relationship between progressive multiple myeloma or macroglobulinaemia and a decrease in BLP is uncertain. Although Spikes *et al.* (1968) have suggested that the serum 'M' component in certain circumstances may interfere with the measurement of BLP, this does not appear to explain the findings in our investigation since in half the responsive patients studied, BLP concentration returned to normal at a time when the paraprotein level had not yet diminished. Furthermore, some but not all of the patients with Bence-Jones myelomatosis and pronounced hypogammaglobulinaemia showed the same pattern of response. Decreased BLP is not generally encountered in malignant neoplastic disease. In fact, increased levels have been observed in many different tumours (Kellen, 1968; Nanava and Tsintsadze, 1961). Ohya (1960) has reported the presence of a cancer specific antigen in the BLP fraction. Further increases in the already abnormally high levels were noted with progression of malignancy (Barclay *et al.*, 1956, 1959). A decreased BLP also does not seem to result from malnutrition. Kellen (1968) found no correlation between nutritional status or the extent of metastases and BLP. Normal levels have moreover been encountered in children suffering from protein calorie malnutrition (Shulman, 1975). These findings suggest that inanition is not responsible for the decreased BLP in multiple myelomatosis and Waldenström's macroglobulinaemia, a conclusion

sion which is supported by finding normal serum albumin concentrations in most of our patients.

Hypolipidaemia, with a decrease in each of the three main plasma lipoprotein fractions, has been described in patients with chronic anaemia (Rifkind and Gale, 1967). There was, however, no correlation between the haemoglobin level and BLP or cholesterol concentrations in our patients either before or after treatment.

The studies of the effects of steroids on plasma lipids and lipoproteins have shown conflicting results. Adlersberg *et al* (1950) showed a significant increase in serum cholesterol in patients receiving steroid therapy. Skanse *et al* (1959) furthermore showed that in short-term steroid therapy initial decrease in plasma lipids and lipoprotein levels was followed by increase affecting both triglycerides and cholesterol. However, significant increase in endogenous triglycerides in asthmatic patients receiving long-term steroid therapy was also demonstrated without changes in cholesterol levels (El-Shaboury and Hayes, 1973; Ghosh *et al*, 1973). Although the presence of direct effect on BLP due solely to steroid therapy could not entirely be discounted in our cases, a rise in BLP occurred only in the responsive patients. Therefore, while the observations reported here suggest that a decrease in serum BLP concentration is associated with progressive multiple myelomatosis or macroglobulinaemia, the reason for the relationship remains obscure.

Two of the other serum protein fractions were significantly altered in our patients with multiple myelomatosis or macroglobulinaemia. Serum transferrin levels were significantly reduced and they returned towards normal in those who responded to therapy. The presence of reduced transferrin and serum iron has been reported in many chronic disorders (Cartwright and Lee, 1971). Serum α_1 -antitrypsin levels were significantly raised before therapy in patients who were not responsive, and in both responders and non-responders after receiving therapy. The total α_1 -antitrypsin is one of the participants of the acute phase reaction (Larson, 1974) and its elevation was not unexpected in our patients.

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