CLINICAL RESEARCH

Increase of oleic acid in erythrocytes associated with malignancies

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Abstract

Total lipid extracts of erythrocyte cell membranes from 60 patients with documented malignancies, 41 patients with various acute and chronic diseases, and 40 healthy subjects were analysed. The results were expressed as ratios of stearic to oleic acid, reflecting the degree of desaturation of stearic acid. The mean ratios for the healthy subjects and controls without cancer were 1.5 (SD 0.27) and 1.45 (0.28), respectively, whereas the ratios for patients with malignancies were consistently lower than the cut off point of 1.0, with a mean of 0.69 (0.15) ($p < 0.001$). The desaturation ratio was also significantly lower ($p < 0.001$) in the group with recurrent tumours (mean 0.75 (0.04)) compared with those with no evidence of recurrent tumours (mean 1.55 (0.27)).

It is suggested that the increased unsaturation (oleic acid) in the circulating erythrocytes may be useful in the diagnosis and postoperative monitoring of patients with cancer.

Introduction

The physical properties of lipids depend on the length of the chain and the degree of desaturation of the constituent fatty acids. A higher content of unsaturated fatty acids increases fluidity of cell membranes, which in turn increases the metabolic rates of many cellular enzymes.^{1,2} It was shown recently that

viruses, interferon, and diphtheria toxin can modulate the saturation of the 18 carbon (C18) fatty acids, especially the conversion of stearic to oleic acid. Interferon,³ diphtheria toxin,⁴ and Sindbis⁵ and Cocksackie viruses⁶ inhibit desaturation of stearic acid, whereas viruses that induce fusogenic syncytia—namely, Newcastle disease virus, herpes simplex type 2, and a putative interferon antagonist³—promote it. Previous work has shown a consistently higher content of oleic acid relative to stearic acid in the peripheral red blood cells of patients with chronic leukaemia compared with that in normal blood cells.⁷ This study assesses the fatty acid saturation index of red blood cell membranes in patients with various solid tumours.

Subjects, materials, and methods

To determine the saturation index of human erythrocytes in states of health and disease, the ratio of stearic to oleic acid was measured in 146 subjects (table). Forty were healthy and formed the reference group. Forty one patients with various non-malignant conditions were the control group. The group with benign tumours included five patients with benign colorectal polyps. The group with cancer comprised 60 patients, who had the following tumours: colorectal (18), pancreatic (10), primary hepatocellular carcinoma (10), cholangiocarcinoma (nine), breast (nine), ovarian (one), squamous cell carcinoma (one), abdominal (one), neuroblastoma (one). The various conditions in the control group included: Crohn's disease (two cases), diverticular disease (two), ulcerative colitis (two), perforated duodenal ulcer (two), cholecystitis (two), sclerosing cholangitis (two), pancreatitis (one), cholelithiasis with jaundice (one), primary biliary cirrhosis (one), varicose veins (five), breast abscess (two), fibroadenosis (eight),

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Saturation index of erythrocytes in states of health and disease

Diagnosis	No of cases	Mean	Range
Reference group of voluntary healthy controls	20	1.57	1.1-2.9
Control group:			
Liver and pancreatic diseases	7	1.05	0.79-1.5
Gastrointestinal diseases	7	1.13	1.01-2.01
Breast diseases	11	1.45	0.96-2.0
Miscellaneous diseases	15	1.67	0.9-2.8
Group with cancer:			
Hepatobiliary	19	0.676	0.2-0.95
Colorectal	18	0.66	0.28-0.96
Pancreatic	10	0.69	0.43-0.89
Breast	9	0.79	0.61-0.95
Miscellaneous	4	0.71	0.59-0.82

FIG 1—Ratio of stearic to oleic acid in 141 patients.

FIG 2—Postoperative monitoring of patients with cancer.

gynaecomastia (one), hernia (three), hypercholesterolaemia (three), chest pain (four).

We also compared the saturation index in a further nine patients, who underwent curative surgery for cancer and were alive and well without any signs of recurrent tumour at the time of taking blood samples, with that in seven patients whose tumours recurred after radical surgical excision of the primary tumours.

A 5 ml blood sample was collected from all patients and centrifuged at 1000 rpm to precipitate red blood cells. Total lipid extraction was carried out with the method described by Apostolov and Barker.⁸ The extracts were analysed by gas liquid chromatography of the fatty acid methyl esters (programmed at 160–260°C; 4°/min) with a glass column (2.1 mm × 2 m inside diameter) packed with a 3% SP-2310/2% SP-2300 in 100/120 mesh Chromosorb W (Supelco Inc). Using this method we could separate C16, C18, C20, and C22 fatty acids. The ratio of stearic to oleic acid was taken as the saturation index. Comparison of variables was made with an unpaired *t* test. Statistical analyses were made by calculating the mean (SD) in each group and the standard error of the differences between means.

Results

We used a 2.1 mm × 2 m column of 3% SP-2310/2% SP-2300 to analyse fatty acids because it separates the C18 fatty acids efficiently. As in previous work with viruses and interferon the only consistent change was in the values of oleic and stearic acid. We took the ratio of stearic to oleic acid as the desaturation index because the normal metabolic flow is the irreversible conversion of stearic to oleic acid by desaturation. Erythrocytes were studied because they are end cells incapable of lipid metabolism.

Figure 1 shows that the mean saturation index for C18 fatty acids was 1.5 (0.27) in the reference group and 1.45 (0.28) in the control group. The saturation index was above 1.0 in all but five patients, of whom three were jaundiced with chronic liver conditions (two with sclerosing cholangitis, one with cirrhosis) in which substantial tissue regeneration or undetected precancerous lesions were suspected. Of the two remaining patients, one had hypercholesterolaemia and the other fibroadenosis of the breast. In all the patients with documented malignancies, however, the index was without exception below 1.0. The mean saturation index in patients with cancer was 0.69 (0.15). The standard error of difference between the means of the ratio of the control and cancer groups was 0.048. The probability of difference between the means was 15.8 ($p < 0.001$).

The comparison of patients with recurrent tumours with those who had been treated successfully and were free from malignant disease was more significant (figure 2). In all patients with recurrent tumours the index was below 1.0 (mean 0.75 (0.04); $n = 7$), whereas in all patients who had successful surgical treatment the index remained above 1.0 (mean 1.55 (0.27); $n = 9$, $p < 0.001$). This group included one patient in whom the index before surgical treatment was 0.6, and four months after radical excision of the tumour the value rose to 1.4.

Discussion

This study shows that in patients with various solid tumours a consistent change is seen in the lipids extracted from red blood cell membranes. A similar change in the saturation index of erythrocytes has been shown in patients with chronic leukaemia.⁷ The relative increase in oleic compared with stearic acid indicates enhancement of the normal process of irreversible desaturation of fatty acids. As no consistent compensatory changes occur in other fatty acids we may assume that an increase in membrane fluidity occurs both in the red cells and in their progenitors. A reversible desaturation of stearic acid in cell membranes has been shown in cells infected with Cocksackie and herpes simplex type 2 viruses. The desaturation effect produced by these viruses was reversible to normal values despite complete cell lysis.⁶ Thus in patients with malignancies a persistent systemic enhancement of the desaturation of stearic acid in red blood cell membranes occurs. We have found (not shown in the results) that the saturation index varies only slightly in specimens collected serially within a week from the same patients. A technician can process 10–15 specimens a day, requiring at least 0.1 ml red blood cells.

The reasons for this persistent change in saturation index are not known. Possibly the tumour secretes a factor or factors that make the cell membranes unstable, not only of the tumour but also of apparently normal circulating cells. We have found that cell membranes of tumours show considerable desaturation with a low saturation index.⁹ A circulating desaturation producing factor might explain some of the systemic effects seen in patients with cancer. Alternatively, the changes in the red blood cell membranes we found may be due to the excessive production of growth factors either by the tumour itself or in response to the tumour. Although evidence exists for increased presence of growth factors in malignancies,¹⁰ little is known about their effects at the cellular level.

The lowest saturation indexes were seen in patients with advanced tumours so that the degree of enhancement of desaturation could reflect the mass of the tumours and the degree of malignancy. Further work should determine whether the effects of the factor causing desaturation can be seen in the volume of a small tumour or in premalignant lesions. Furthermore, patients with chronic obstructive jaundice had a low

saturation index. This may have been due to impaired metabolism of lipids, substantial regeneration of tissue within the liver, or undetected precancerous lesions, especially in the patient with cirrhosis.

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IgM and IgG antibodies to human T cell lymphotropic retrovirus (HTLV-III) in lymphadenopathy syndrome and subjects at risk for AIDS in Italy

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Abstract

A study was performed to assess the prevalence of specific antibodies to human T cell lymphotropic retrovirus (HTLV-III) in patients with lymphadenopathy syndrome, patients with the acquired immune deficiency syndrome (AIDS), and those at risk of AIDS. Serum samples were obtained from these groups and from healthy controls in selected cities in Italy, and antibodies to HTLV-III were measured by immunofluorescence assay and, in a few patients, by Western blotting. In addition, IgM antibody values were measured in 82 of those positive for HTLV-III. Altogether, 235 out of 320 patients with lymphadenopathy syndrome had antibodies to HTLV-III, the proportions being highest in haemophiliacs, homosexuals, and drug addicts from Rome; 11 out of 12 patients with AIDS had antibodies; 78 out of 439 subjects at risk for AIDS had antibodies; and six out of 30 patients with lymphadenopathy syndrome and positive for HTLV-III antibodies and nine of 52 patients at risk of AIDS had a detectable titre of IgM.

HTLV-III is widespread in groups at risk of AIDS in

Italy, and antibodies to HTLV-III are highly prevalent in patients with lymphadenopathy syndrome. A higher proportion of drug abusers were positive for antibodies than in previous studies. HTLV-III "infection" would appear to be spread mainly in compromised hosts, as none of the controls were positive for antibodies.

Introduction

Acquired immune deficiency syndrome (AIDS) and lymphadenopathy syndrome, prevalent in homosexuals, drug abusers, and haemophiliacs, are aetiologically related to two retroviruses—namely, lymphadenopathy virus (LAV)¹ and human T cell lymphotropic retrovirus (HTLV-III).² In the past two years cases of lymphadenopathy syndrome have increased in Italy, but only 29 cases of AIDS have been recorded.³ We report an epidemiological study investigating the prevalence of specific antibodies to HTLV-III in patients with AIDS and lymphadenopathy syndrome and subjects at risk for AIDS in Italy.

Patients and methods

Serum samples were obtained from patients and healthy subjects in Rome, Milan, Florence, Naples, Cagliari, Verona, Turin, Genoa, Viterbo, and Catanzaro.

Lymphadenopathy syndrome—Criteria for selecting patients with lymphadenopathy syndrome were those established by the CDC-NIH working group. In addition to unexplained lymphadenopathy affecting two or more extrainguinal sites and persisting for more than three months all patients had two or more other symptoms, such as fatigue, muscle pain, night sweats, weight loss, and moderate fever. They also had two or more of the following abnormalities: cutaneous anergy, lymphopenia, inversion of the OKT4:T8 ratio, increased immunoglobulin concentrations, and raised antibody titre to cytomegalovirus or Epstein-Barr virus or both. The overall population consisted of 320 patients with lymphadenopathy syndrome, of whom 298 were drug addicts, 16 homosexuals, and six haemophiliacs (see table). In 30 drug abusers and five homosexuals serum samples had also been collected one to two years before, when the patients were symptom free.

AIDS—Serum samples were collected from 12 patients with AIDS.

Subjects at risk for AIDS and lymphadenopathy syndrome (see table)—Serum samples were collected from 70 homosexuals or bi-

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