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Altered Platelet Stearic to Oleic Acid Ratio in Malignancy

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Alteration of lipid metabolism associated with malignant disease is well-documented and some studies have suggested a reduced stearic to oleic acid ratio occurs in erythrocytes in cancer patients. In this study, the fatty acid composition was measured in platelets, which are capable of lipid synthesis and have a much shorter lifespan. While demonstrating any malignancy related change in the platelet stearic to oleic acid ratio the study aimed to assess whether it could be of value as a tumour marker. Patients with active malignancy (n=46) had a lower ratio of stearic to oleic acid than those with malignant disease in clinical remission [mean (S.D.) 1.08 (0.22) vs. 1.26 (0.30), P<0.01], and 22 healthy controls [1.29 (0.24), P<0.001]. However in a group of 17 patients with chronic, non-malignant diseases the ratio was also lower than in normal controls and similar to that seen in the active malignancy group [0.97 (0.29)]. Thus while a reduction in platelet stearic to oleic acid ratio was found in active malignancy, it is not specific to neoplastic disease.
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INTRODUCTION

ALTERATION OF lipid metabolism in patients with malignant diseases has long been recognised [1]. Any change in plasma lipid composition in such a situation has the potential to affect membrane lipids of blood cells as a result of direct exchange of phospholipid molecules between plasma and membrane [2]. Wood *et al.* [3] studying erythrocyte membrane lipids found a marked reduction in the ratio of stearic to oleic acid present in patients with cancer. Subsequent reports by other workers looking at this fatty acid ratio in groups of patients with malignant diseases have been less encouraging showing either no difference between patient and control groups [4, 5] or too great an overlap between groups for the ratio to have any discriminatory value [6, 7]. More recently Wood and his co-workers have repeated their findings showing again a discrete, though less pronounced separation between the lowered ratios found in patients with malignancy and those found in control groups [8]. However they also found that the erythrocyte ratio did not return to normal in the majority of patients who had undergone apparently successful surgical treatment for bowel cancer and concluded that the erythrocyte ratio was of no value in postoperative follow-up of such patients.

In contrast to erythrocytes, platelets possess the enzymatic capacity for *de novo* synthesis of fatty acid and phospholipid molecules [9, 10] which can then be incorporated into their membrane structure. In addition platelet membranes have been shown to be capable of selective plasma lipid uptake [11]. Normal erythrocytes survive 120 days after leaving the bone marrow whereas the normal platelet lifespan is much shorter at 7–10 days. This enhanced potential for membrane lipid modification, and the rapid population turnover suggest that circulating platelets may be more responsive than erythrocytes to any tumour-related factors which influence lipid metabolism. The current study sought to measure any difference in the platelet stearic to oleic acid ratio in patients with malignant diseases and to assess the value of this ratio as a marker of active malignancy.

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PATIENTS AND METHODS

Blood samples were obtained from 74 patients attending an out-patient Medical Oncology Unit. Active malignant disease was present in 46 patients (23 males) aged 25–79 years (mean 55) with a range of tumours: breast (11), bronchial (15), non-Hodgkin lymphoma (5), myeloma (4), hepatocellular (3), testicular (2), and others (6). 28 patients (22 males) aged 17–65 years (mean 45) were considered to be in clinical remission following successful treatment of malignant tumours: breast (5), testicular (9), non-Hodgkin lymphoma (5), bronchial (2), Hodgkin's lymphoma (2) and others (5). Samples were also taken from 22 healthy colleagues (14 males) aged 31–59 years (mean 48), and 17 patients (10 males) aged 40–70 years (mean 59) with non-malignant, chronic diseases: chronic obstructive airways disease (6), rheumatoid arthritis (4), advancing chronic renal failure (3), chronic asthma (2), sarcoidosis (1) and long-standing quadraplegia (1). Of the latter group 12 were attending hospital as out-patients at the time of testing and the remaining 5 were short stay in-patients.

A 10 ml sample of venous blood was collected from each subject into a tube containing ethylene diamine tetracetic acid (EDTA). All samples were transported to the laboratory and the platelets separated from plasma within 4 h. The blood was centrifuged at 200 *g* for 15 min at room temperature to obtain a supernatant of platelet rich plasma. This was carefully transferred to a second EDTA tube by means of a plastic pipette. Further centrifugation of the platelet rich plasma at 2000 *g* for 15 min at room temperature gave a small platelet pellet. The platelet-poor plasma was decanted and the pellet resuspended in 4 ml of tris-EDTA buffer solution. After a further centrifugation

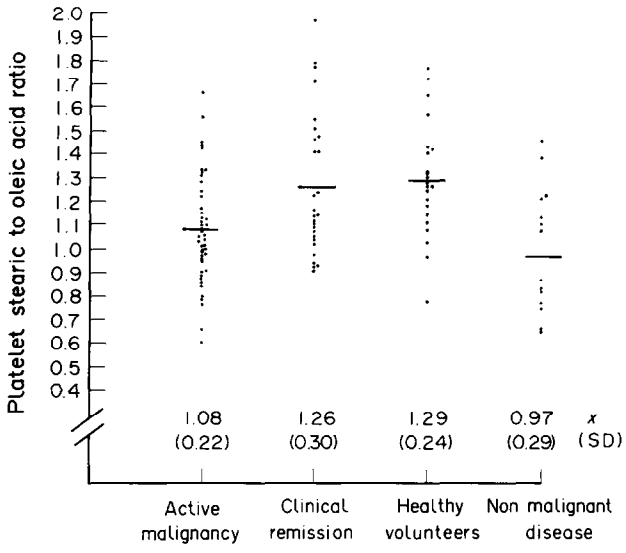

Fig. 1. Platelet stearic to oleic acid ratios in 46 patients with active malignancy, 28 patients in clinical remission following successful treatment of malignant disease, 22 healthy volunteers and 17 patients with chronic non-malignant diseases.

and washing stage at 1000 *g* the platelet pellet was resuspended in 1 ml of EDTA buffer solution. The lipid content was extracted using a modification of a technique originally described by Broekhuyse [12]. This involved sequential mixing with methanol, chloroform and hydrochloric acid to a final 2:1 mixture of chloroform and methanol. During this extraction and all subsequent stages of lipid analysis, butylated hydroxytoluene was present in the solvents to help minimise lipid auto-oxidation. After standing overnight the partitioned chloroform layer was carefully withdrawn into a flask and the solvent evaporated under vacuum. The extracted lipid was refluxed with molar potassium hydroxide in 95% ethanol for 2 h to allow saponification. After dilution to three times volume with distilled water the mixture was acidified with concentrated sulphuric acid. The fatty acids now present were extracted into diethyl ether, removed into a potassium hydroxide layer, reacidified and then re-extracted into diethyl ether. After washing and careful removal of traces of moisture the fatty acids were refluxed in sealed tubes with 5% methanolic hydrochloric acid for 2 h at 95°C. The more volatile fatty acid methyl esters thus formed were presented in 10 μl of hexane for analysis by gas liquid chromatography. A Pye Unicam 4500 series machine (Pye Unicam Ltd, Cambridge) with a sil 5 column (Chromopack UK Ltd, London) was run at 195°C for 6 min increasing by 6°C per min to 210°C. The separated peaks of the fatty acid methyl esters were identified by their relative retention times. Known standards of C16 to C22 fatty acid methyl esters, including stearic (C18:0) and oleic (C18:1) acid esters, were run with each batch of samples. The area under each peak, and the relative ratios were calculated using a Spectra Physics integrator.

RESULTS

The values found in the four subject groups are shown in Fig. 1. Analysis by the Student's *t*-test showed a significantly lower ratio of platelet stearic to oleic acid in patients with active malignancy compared with those in clinical remission (difference = 0.18, 95% confidence interval (CI) 0.06–0.3, $P<0.01$) and healthy volunteers (difference = 0.21, 95% CI 0.09–0.33, $P<0.001$). The ratios found in the healthy volunteer and remission groups were similar. Ratios in the group of patients with non-malignant chronic diseases were also reduced to a similar extent to those in the active malignancy group and were significantly lower than those in the healthy volunteer group (difference = 0.32, 95% CI 0.15–0.49, $P<0.001$).

DISCUSSION

The results show a significant reduction in platelet membrane stearic to oleic acid ratio in patients with active malignancy. Following apparently successful treatment this platelet lipid ratio was indistinguishable from normal. The observed lipid modification is not however specific to the malignant state as it was also found in patients with non-malignant, debilitating, chronic illness. The extent of the reduction in platelet stearic to oleic acid ratio in a group of patients with a variety of active malignancies was less marked than that found in erythrocytes by Wood *et al.* [3, 8]. The clear separation between malignant and control groups which they had shown [3, 8] was neither seen in the current platelet study nor found by a number of other workers seeking to reproduce the findings in erythrocytes. Taylor *et al.* [7] found patients with bronchial carcinoma to have a small, but significant, reduction in stearic to oleic acid ratio although many individual values fell within the range seen in healthy controls. Søreide *et al.* [6] looking at a variety of malignant tumours, and Thomas *et al.* [5] studying breast disease failed to show any significant difference between patient groups with malignant and non-malignant conditions.

The mechanism responsible for producing the observed reduction in stearic to oleic acid ratio in platelets remains speculative. Effects of altered body composition, humorally active tumour factors, anti-neoplastic and other drug therapies and particularly changes in nutritional state may act singly or in any combination to produce direct or indirect effects on membrane lipid composition. The similarity in lipid pattern in the ill, non-malignant group suggest an important influence of those non-specific factors associated with debility. Søreide *et al.* [6] have suggested that the altered ratio (in erythrocytes) is simply age-dependent, claiming that previous studies had compared malignant disease patients with younger controls. However, those with active malignant disease in the present study were more closely matched for age than in some of the earlier studies, and there was no correlation between age and fatty acid ratio across the study subjects as a whole ($r = 0.092$, $P = 0.825$).

In summary, a method has been described for measuring platelet lipid composition, and a significant reduction in stearic to oleic acid demonstrated in a group of patients with active malignant disease compared to healthy controls and patients with malignant disease in clinical remission. The reduction was not specific for malignancy, being found in other chronic illness, and it was not sufficiently marked to have any diagnostic potential. However, serial studies within patients may yet provide an early indication of individual responses to antitumour therapy and further study is required into the nature and properties of the tumour-related effects on lipid metabolism and platelet lipid composition.

1. Haven FL, Bloor WR. Lipids and cancer. *Adv Cancer Res* 1956, **4**, 238–314.
2. Reed CF, Murphy M, Roberts C. Phospholipid exchange between plasma and erythrocytes in man and dog. *J Clin Invest* 1968, **47**, 749–760.
3. Wood CB, Habib NA, Thomson A, *et al.* Increase of oleic acid in

erythrocytes associated with malignancy. *Br Med J* 1985, **291**, 163–165.
4. Neoptolemos JP, Clayton H, Heagarty AM, *et al.* Dietary fat in relation to fatty acid composition of red cells and adipose tissue in colorectal cancer. *Br J Cancer* 1988, **58**, 575–579.
5. Thomas BS, O'Dea J, Fentiman IS. Erythrocyte membrane stearic and oleic acid ratios in breast cancer. *Br J Surgery* 1988, **75**, 1078–1079.
6. Søreide O, Bakken AM, Lygre T, Farstad M. Inadequacy of oleic acid in erythrocytes as a marker of malignancies. *Br Med J* 1987, **294**, 548.
7. Taylor A, Manche A, Wilson I, Watson D, Pandov H, Lawson N. Erythrocyte fatty acid profiles in patients with bronchogenic carcinoma. *Ann Clin Biochem* 1987, **24**, 604–607.
8. Kelly SB, Millar J, Wood CB, Williamson RCN, Habib NA. Erythrocyte stearic acid desaturation in patients with colorectal carcinoma. *Dis Col Rect* 1990, **33**, 1026–1030.
9. Majerus PW, Smith MB, Clamon GH. Evidence for a complete fatty acid synthesizing system. *J Clin Invest* 1969, **48**, 156–163.
10. Lewis N, Majerus PW. *De novo* phospholipid synthesis and the effect of thrombin on the pattern of synthesis. *J Clin Invest* 1969, **48**, 2118–2123.
11. Joist JH. Phospholipid transfer between plasma and platelets *in vitro*. *Blood* 1976, **48**, 199–211.
12. Broekhuyse RM. Quantitative two dimensional thin layer chromatography of blood phospholipids. *Clin Chim Acta* 1969, **23**, 467.

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Levamisole plus 5-Fluorouracil Inhibits the Growth of Human Colorectal Xenografts in Nude Mice

Robert Van Ginckel, Wim Distelmans, Marc De Brabander, Myriam Callens, Boudewijn Janssens, Els Jagers, Luc Wouters, Roland De Coster and Paul A.J. Janssen

Fragments of human colorectal adenocarcinomas were inserted under the renal capsule of nude mice. The growth of these tumour grafts was significantly inhibited by the combination of 5-fluorouracil (5-FU) and levamisole. An alternating regimen of levamisole 2.5 mg/kg and 5-FU 20 mg/kg decreased the size of tumour implants by 33–59% and/or increased the number of macroscopically disappeared fragments in the combined group compared with ineffective monotherapy with saline, levamisole or 5-FU. This model could be valuable for investigating the mechanism of action of levamisole and to evaluate the effects of this adjuvant therapy in other oncological settings.
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INTRODUCTION

THE IMMUNOMODULATING properties of levamisole have been extensively investigated in neoplastic disease [1–4]. In several studies, the compound was shown to be effective on slowly growing tumours particularly in combination with cytoreductive therapies (surgery, radiotherapy or chemotherapy) and to exert antimetastatic activities. The critical dose-dependency and timing of levamisole administration frequently hampered the beneficial outcome [5–9]. Due to the difficulties in establishing efficacious levamisole treatment, together with reported granulocytopenia [4], the possible benefit of immunoadjuvant therapy with levamisole was regarded with scepticism.

Recently, a revival of interest in the compound was triggered by a large scale intergroup clinical trial, which showed that adjuvant treatment with levamisole and 5-fluorouracil (5-FU) after surgery is beneficial (30% increase in 5-year survival) in patients with node-positive colon cancer [10]. Here we describe the results with levamisole, 5-FU and their combination on the growth of human colorectal adenocarcinoma grafted under the renal capsule of nude mice.

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MATERIALS AND METHODS

Colorectal carcinomas of untreated patients, obtained under sterile conditions at surgery, were immersed in minimal essential medium (MEM–REGA) supplemented with 2 μmol/l glutamine