Evaluation of the erythrocyte-UFA (E-UFA) mobility test for the diagnosis of multiple sclerosis

J CUYPERS AND H REDDEMANN

From the Department of Neurology, Klinikum Steglitz, Berlin, West Germany

SUMMARY The absolute electrophoretic mobility of red blood cells was examined before and after the addition of linoleic acid. The determinations were performed with a Zeiss cytopherometer. The blood was taken from 22 patients with multiple sclerosis in different phases of their disease. A group of 17 patients with other neurological diseases and three apparently healthy subjects served as controls. Our results show that the mobility of red blood cells was either accelerated or inhibited in the presence of linoleic acid, both in the multiple sclerosis patients as well as in the control group. The increase or inhibition of the absolute mobility was small and randomly distributed. The value of this so-called E-UFA test (erythrocyte-unsaturated fatty acid) as a specific diagnostic test for multiple sclerosis is not confirmed by our results.

A precise diagnosis of multiple sclerosis would be of great importance for research and clinical management, for at present it is a matter of probability and exclusion. Recently Field *et al*¹ claimed to have found a diagnostic test for multiple sclerosis which was said to be valid in each phase of the disease and extremely specific. In this test the electrophoretic mobility of erythrocytes of patients with multiple sclerosis (MS) is inhibited in the presence of linoleic acid. The test is based on results which indicate an anomaly of fatty acid metabolism producing changes both in myelin composition and in the lipid structure of other cells, for instance red blood cells.²⁻⁵ Reported results on the value of this so-called E-UFA (erythrocyte-unsaturated fatty acid) test have been controversial.⁶⁻⁹ We therefore examined this particular test and the following paper describes our experiences and results.

Material and method

Fresh red blood cells (RBC) were prepared from 20 MS patients in different stages of the disease. Only those patients who could be judged as definite, using the criteria of McAlpine, were selected.¹⁰ Three apparently healthy persons and 19 patients with other neurological disorders (OND) (cerebrovascular

accidents, Parkinsonism, brain tumor, lumbar disc prolapse and heredity ataxia) served as controls. All subjects, who agreed to participate in the test, told us they did not use a diet containing a high quantity of polyunsaturated fatty acids.

The method used was essentially the same as described by Field.¹ Red blood cells were prepared by defibrination of 15 ml of fresh blood with glass beads under sterile conditions. The further procedure was carried out in tubes and pipettes highly purified for one day in *n*-HCl and bidistilled water, but not sterilised; sterilisation did not prove to be of any importance in preliminary experiments. The red cells were washed three times in sterile 0.9% saline and then once in Hanks Medium 199 and centrifuged at 400 *g* after each step. Finally they were suspended in this medium at a concentration of 2.8×10^7 cells/ml and processed immediately.

The absolute electrophoretic mobility was measured in a Zeiss cytopherometer. The current used was 7.4 mA. The time which one erythrocyte needed for going ahead and back one field in the ocular measuring grid ($=32 \mu$) was measured with a stopwatch. Forty cells were measured in each blood suspension, and the mean value and standard deviation were calculated. A measurement was only performed when there was vertical sedimentation of erythrocytes. This was often difficult and sometimes impossible, especially in the beginning. The reason for this "drift" is unknown. The absolute mobility was calculated by the following formula:

$$\text{mobility} = \text{speed/field strength} = \frac{3136}{t \times 7.4 \times \rho}$$

(*t*=time in seconds to move 32 μ , ρ =specific resistance of the medium).

Address for reprint requests: Dr med Jochen Cuypers, Department of Neurology, Klinikum Steglitz of the Free University of Berlin, Hindenburgdamm 30, D 1000 Berlin 45, West Germany.

Accepted 16 July 1980

The specific resistance of our medium was determined in the conductolyser and had a value of about 70 (± 2) Ω per cm. The determinations were carried out blindly, that is the examiner did not know whose blood he was processing. After determining the mobility of the erythrocytes without linoleic acid, a consecutive redetermination was done by adding 20 μ l (equivalent to 0.16 g/l) of highly purified linoleic acid to the erythrocyte suspension. We chose this concentration on the basis of a suggestion made by Field, who told us that the higher concentration would bring better results. The temperature of the measuring chamber remained constant at 25°C.

In the second half of the experiments, detergents (Tween 80, Triton) were added to the suspension in order to improve the solubility of linoleic acid. In those cases determinations were performed (or the attempt was made to do so) with and without detergents.

Results

1 MS Patients

The values of each patient blood either with or without linoleic acid (LA) are shown in table 1. The addition of LA led to rather irregular—positive or negative—deviations (p not significant using the Wilcoxon test). The range was from -9.94 to +6.78% with a mean value of -0.92%.

Most determinations were performed two or

Table 1 MS patients without linoleic acid (= control) and with linoleic acid: mean value and SD of the absolute mobility of red blood cells (μ /s/V/cm)

Pat number	Absolute mobility in μ /s/V/cm		Change in absolute mobility in %	Remarks
	Control	Linoleic acid		
1	0.822 (0.032)	0.775 (0.038)	-5.72	Stable MS
3	0.784 (0.030)	0.818 (0.047)	+4.34	Acute MS
4	0.805 (0.039)	0.725 (0.029)	-9.94	Acute MS
5	0.740 (0.035)	0.734 (0.038)	-0.81	Stable MS
6	0.735 (0.038)	0.741 (0.036)	+0.82	Acute MS
7	0.735 (0.036)	0.706 (0.031)	-3.95	Stable MS
8	0.734 (0.042)	0.722 (0.032)	-1.6	Stable MS
9	0.728 (0.036)	0.763 (0.031)	+4.8	Chron prog MS
10	0.791 (0.037)	0.771 (0.045)	-2.5	Acute MS
14	0.804 (0.047)	0.791 (0.038)	-1.6	Acute MS
15	0.756 (0.040)	0.734 (0.037)	-2.9	Stable MS
16	0.761 (0.040)	0.719 (0.038)	-5.52	Stable MS
19	0.724 (0.033)	0.762 (0.035)	+5.2	Stable MS
20	0.756 (0.030)	0.747 (0.040)	-1.2	Chron prog MS
22	0.758 (0.042)	0.738 (0.041)	-2.6	Stable MS
23	0.783 (0.031)	0.750 (0.029)	-4.21	Stable MS
26	0.865 (0.046)	0.868 (0.042)	+0.35	Chron. MS
27	0.797 (0.035)	0.870 (0.049)	+1.63	Chron. MS
29	0.794 (0.031)	0.768 (0.031)	-3.27	Acute MS
37	0.767 (0.046)	0.819 (0.054)	+6.78	Acute MS
40	0.785 (0.047)	0.786 (0.031)	+0.13	Acute MS
43	0.787 (0.034)	0.798 (0.038)	+1.38	Acute MS

Table 2 Patients with other neurological diseases and healthy subjects without linoleic acid (= control) and with linoleic acid: mean value and SD of the absolute mobility of red blood cells (μ /s/V/cm)

Pat number	Absolute mobility in μ /s/V/cm		Change in absolute mobility in %	Remarks
	Control	Linoleic acid		
2	0.794 (0.023)	0.791 (0.023)	-0.38	Polyneuropathy
11	0.741 (0.039)	0.734 (0.037)	-0.94	Cer ischaemia
12	0.778 (0.049)	0.755 (0.036)	-3.0	Parkinsonism
13	0.724 (0.026)	0.759 (0.039)	+4.8	Stroke
21	0.746 (0.033)	0.744 (0.030)	-0.27	Epilepsy
24	0.834 (0.046)	0.858 (0.039)	+2.88	Arteritis cranialis
28	0.774 (0.034)	0.767 (0.034)	-0.9	Stroke
30	0.751 (0.030)	0.759 (0.041)	+1.07	Crohn's disease
34	0.871 (0.040)	0.844 (0.052)	-3.10	Parkinsonism
35	1.012 (0.055)	0.905 (0.056)	-10.5	Cer vas disease
36	0.967 (0.063)	0.924 (0.048)	-4.45	Stroke
38	0.771 (0.038)	0.738 (0.033)	-4.28	Parkinsonism
39	0.820 (0.046)	0.772 (0.033)	-5.85	Parkinsonism
41	0.761 (0.040)	0.778 (0.041)	+2.23	Parkinsonism
42	0.808 (0.034)	0.829 (0.047)	+2.60	Parkinsonism
17	0.790 (0.041)	0.766 (0.040)	-3.03	Healthy
18	0.783 (0.043)	0.761 (0.036)	-2.80	Healthy
33	0.885 (0.055)	0.804 (0.050)	-9.15	Healthy
44	0.830 (0.042)	0.792 (0.040)	-4.80	Friedreich's ataxia
45	0.817 (0.043)	0.765 (0.038)	-6.80	Hereditary ataxia

three times, either the same day or one day later. These values (which are not mentioned in the tables) revealed the same tendency, that is the deviation remained positive or negative. In some cases, however, it changed. Detailed analysis of the values showed no correlation to certain stages or subtypes (acute or stable, relapsing or chronic).

2 Control group (normal persons and patients with other neurological disorders)

The values are shown in table 2. LA produced an acceleration in five of 20 cases and an inhibition of the absolute mobility in the remaining cases (p = significant at the 5% level using the Wilcoxon test). The range of this deviation was from -10.5 to +4.8 with a mean value of -2.33%. The last two patients in table 2 suffered from a hereditary ataxia (Friedreich's ataxia and Marie's ataxia). Anomalies of erythrocytes have also been reported in these patients.¹¹ The inhibition found in these two patients was in the same range as the other cases. When comparing the relative deviations produced by LA in the two groups it can be seen that 11 of 22 cases in the MS group and 15 of 20 cases in the control group showed an inhibition of the absolute mobility. Using the Wilcoxon test, this difference

was significant at the 5% level, but the tendency was just the opposite of what was expected.

Discussion

The E-UFA test is based on a variety of observations which indicate an involvement of fatty acids and especially of polyunsaturated fatty acids (PUFA) in multiple sclerosis.^{2-5 12-15} Agranoff and Goldberg³ and Galli² showed that deficiency of PUFA in food led to permanent changes in myelin composition, which in turn could result in reduced resistance to a pathogenic factor. Other authors,¹² proposed an inborn error of fatty acid metabolism in MS patients. Gul,⁴ Tsang⁵ found a significant decrease of PUFA in phospholipids of erythrocytes, platelets and lymphocyte membranes in MS patients. There are also several reports of a decreased level of PUFAs in MS blood,¹⁵ normal levels, however, could also be shown.¹⁶ The findings of Love,¹⁷ who observed a decrease in PUFA blood concentrations in patients with acute non-neurological diseases, indicate that this may be an unspecific effect.

These results, the validity of which cannot be discussed here, were attractive in suggesting a predisposing factor which might possibly be treated. They led to therapeutic trials with linoleic acids.¹⁸⁻²⁰ The results, however, were disappointing as they showed no or only a slight beneficial effect of long-term intake of LA on the course of the disease. This PUFA deficiency was also used as a diagnostic test and first reports were very encouraging. Our own results, however, with the E-UFA test, in agreement with those of others⁷⁻⁸ do not favour this procedure in the diagnosis of MS. The percentage deviations in form of an acceleration or deceleration of the erythrocyte in the electrical field were small and insignificant. They were irregular, rather randomly distributed and in a range considered by experienced groups to be normal for this method. We could not even see a tendency for erythrocytes to decelerate in MS patients or to accelerate in controls; indeed, the results tended the opposite way.

Because our primary aim was to examine the validity of the method we dispensed with the use of archidonic acid and oleic acid. Another reason was that, according to Field, these two fatty acids rapidly haemolysed the erythrocytes; determinations of the mobility thus becoming difficult. In our results the absolute mobility of the erythrocytes (at a current strength of 7.4 mA) was approximately 20–30% lower than that

usually described in the literature.²¹ A technical examination of our cytopherometer did not show any defect, so that we do not know the reason for this lower value. The absolute mobility of the erythrocytes was in the same range in all determinations throughout the entire experimental period. The standard deviation was still lower than that reported in the literature. This shows that the cytopherometer did function accurately. We also determined whether a higher absolute mobility, approximately in the range of that reported in the literature (= about 1.1 mA/U/s) had any influence on our results. At first we measured the absolute mobility of the erythrocytes on our cytopherometer at such a high current strength (~ 9 mA) that it increased to about 1.1 mA/U/s. The values did not show any alterations compared to those obtained at the lower absolute mobility. Furthermore, we made determinations on another cytopherometer (kindly put at our disposal by Dr Weinhold, Bundesgesundheitsamt Berlin). Here the absolute mobility actually did reach values of 1.1 mA/U/s at a current strength of 7.4 mA. After addition of linoleic acid to the erythrocyte suspension the same irregular and slight deviations could be seen as with our apparatus. From this we conclude that the absolute mobility—at least in the range we observed—had no influence on the test results.

Linoleic acid was rapidly and apparently completely dissolved in absolute alcohol. However, on adding this solution to the erythrocyte suspension linoleic acid appeared in the form of droplets on the surface of the watery solution. Determinations with C₁₄-LA showed that LA was, at the concentration used, only dissolved to 10% in the watery erythrocyte suspension. We do not know if this low solubility had any influence on the results. The use of other solvents (for example acetone) gave rise to difficulties because of the rapid haemolysis of the erythrocyte. Therefore we tried to improve the solubility of linoleic acid by the addition of detergents. But even the use of detergents which should not lead to haemolysis, regularly did so. Finally we would emphasise that the use of the cytopherometer is often marred by rather “capricious quirks” of the apparatus. Reproducible results can only be obtained by a long training period and very exact handling.

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