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Note

Influence of corticosteroid therapy on the fatty acid composition of serum lecithins monitored by means of thin-layer and gas—liquid chromatography

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One of the most provoking problems in clinical neurology is the elucidation and treatment of multiple sclerosis. According to some studies [1—6], symptoms of polyunsaturated fatty acid deficiency in multiple sclerosis patients were found.

In this preliminary trial we have studied the fatty acid pattern in serum choline phosphoglycerides (CPG) in patients other than multiple sclerosis patients. Comparison of untreated and prednisone-treated patients suffering from various diseases could elucidate whether corticosteroid therapy has any effect on the fatty acid composition of serum lecithins. CPG was studied as it is a principal phospholipid class of mammalian tissue [7] which is easy to isolate and transmethylate [8]. From the methodological point of view, the most suitable method in following the phospholipid fatty acid pattern is a combination of thin-layer chromatography (TLC) and gas—liquid chromatography (GLC). In this study a modified procedure of Karlsson et al. [8] was used.

MATERIAL

Group A: five women with progressive polyarthritis, mean age 56 years, not treated with corticosteroids. Group B: eleven patients (two men and nine women, mean age 61.3 years) treated for years with prednisone (10—20 mg/day). Group C: eight patients (four men and four women, mean age 58 years) with osteoarthrosis (coxarthrosis, gonarthrosis) treated with daily doses of 10—20 mg of prednisone for several months. Group D: eight asthmatics treated for several weeks by daily doses of 20—50 mg of prednisone. Group E: five patients of group D studied 1.5 h after parenteral application of 0.25 mg of adrenocorticotropin (ACTH). Group F: twelve healthy blood donors (men, mean age 28 ± 5 years, 178 cm, 81 kg).

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METHODS

For GLC a Perkin-Elmer F-30 gas chromatograph equipped with dual flame ionization detectors and an electronic integrator SIP 1 was used. A Chromaton N-AW DMCS, 0.125—1.16 mm (No. 751194, Lachema, Brno, Czechoslovakia) coated with 15% DEGS. The carrier gas was nitrogen at a flow-rate of 40 ml/min; injection and detection temperature was 250° C.

For TLC silica gel plates (Fertigplatten Merck No. 5721; Merck, Darmstadt, G.F.R.) were used in a saturated chamber. The mobile phase was choroform—methanol—water—acetic acid (65:35:5:1, v/v).

For sample preparation, venous blood was taken after 12 h of fasting and the serum stored at −20° C until analysis. Extraction of lipids was performed with chloroform—methanol (1:1, v/v) [8]. Total lipid extract corresponding to 0.1 ml of serum was applied on silica gel plates and separated in chloroform—methanol—water—acetic acid (65:35:5:1) at room temperature (Fig. 1). The

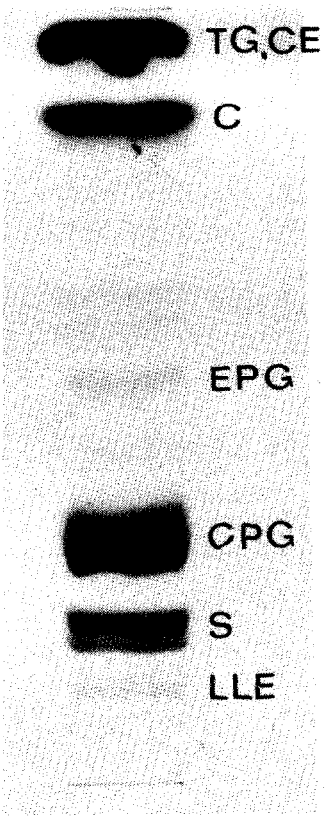

Fig. 1. TLC of total serum lipid extract (preparative, TLC, 0.1 ml of serum). C = Cholesterol, CE = cholesterol esters, TG = triglycerides, EPG = ethanolamine phosphoglycerides, CPG = choline phosphogylcerides, S = sphingomyelins, LLE = lysolecithins. Mobile phase: chloroform—methanol—water—acetic acid (65:35:5:1, v/v), silica gel G (Merck Fertigplatten No. 5721); detection copper acetate reagent.

CPG fraction after visualization with bromphenol blue were scraped off and dried in a dessicator over phosphorus pentoxide for 48 h. Transmethylation was done with 2 ml of 0.2 mol/l sodium methoxide in methanol at 37°C for 60 min. The fatty acid methyl esters (FAME) after acidification were extracted into petroleum ether, dried under N_2 and redissolved in 5 μl of petroleum ether, of which 2 μl were analysed by GLC (isothermal at 180°C). The peaks were identified by comparing their retention times with those of pure FAME (Applied Science Labs., State College, PA, U.S.A., and Serva, Heidelberg, G.F.R.). Values are given in molar per cent (18:1 = 1).

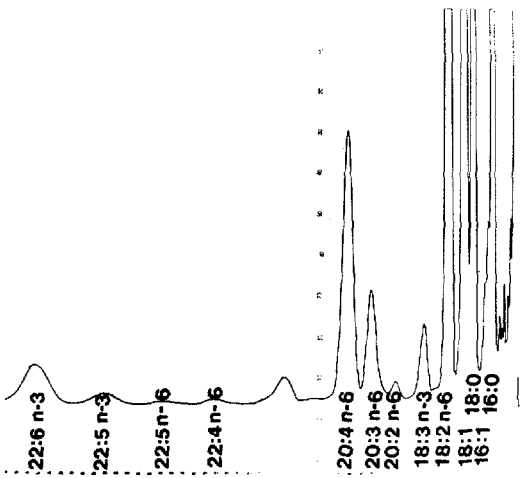

Fig. 2. GLC of serum lecithin FAME (see text for experimental details).

RESULTS

Mean values and standard deviations (S.D.) of FAME in serum CPG are presented in Table I.

Group A (non-prednisone-treated patients with progressive polyarhtritis) shows in comparison with healthy blood donors (group F) a significant decrease in linoleic acid (18:2, $n-6$) and docosahexaenoic acid (22:6, $n-3$), an increase in oleic acid (18:1) and surprisingly also arachidonic acid (20:4, $n-6$).

In all groups (B, C, D, E) treated with prednisone the levels of linoleic, arachidonic and docosahexaenoic fatty acids seem to be normalized. The amount of linoleic acid still remains lower than in healthy blood donors (group F).

There were no statistically significant differences in serum CPG FAME pattern in asthmatics under chronic treatment with prednisone (group D) and in those patients (group E) studied 1.5 h after ACTH application.

The level of polyunsaturated fatty acids in serum lecithins seems to be influenced by prednisone in chronic experiments. An acute bolus of ACTH (and endogenous steroids) evokes no apparent change in serum CPG FAME composition.

TABLE I

FATTY ACID METHYL ESTERS (FAME) OF SERUM LECITHINS IN NON-PREDNISONE-TREATED PROGRESSIVE POLYARTHRITIS (PAP) PATIENTS AND IN PREDNISONE-TREATED PATIENTS

FAME	PAP $\bar{x}$ ± S.D., $n = 5$		Treated with prednisone								Healthy blood donors $\bar{x}$ ± S.D., $n = 12$	
			PAP $\bar{x}$ ± S.D., $n = 11$		Osteoarthroses $\bar{x}$ ± S.D., $n = 8$		Asthmatics $\bar{x}$ ± S.D., $n = 8$		Asthmatics + ACTH $\bar{x}$ ± S.D., $n = 5$			
16:0	30.30	2.37	33.07*	1.86	31.11	1.66	32.60*	2.32	33.09*	1.77	31.63	1.34
16:1	2.20	1.03	1.13*	0.60	1.70	0.40	1.31	0.56	1.57	0.88	0.71**	0.28
18:0	15.28	0.96	16.81*	1.66	15.87	1.48	14.90	0.99	14.96	0.79	15.31	0.49
18:1	14.72	1.51	14.93	1.00	14.23	1.83	13.80	0.43	13.70	0.44	13.56**	0.43
18:2 (n—6)	18.90	1.21	20.79*	2.39	21.20*	1.43	21.23*	1.59	21.43*	1.32	23.42**	1.50
18:3 (n—3)	0.97	0.85	0.53	0.11	0.46	0.13	0.47	0.09	0.59	0.19	0.45	0.05
20:2 (n—6)	0.89	0.95	0.39	0.51	0.37	0.16	0.52	0.29	0.45	0.16	0.36	0.04
20:3 (n—6)	4.20	0.76	2.61*	0.91	3.23	0.54	2.93*	0.64	2.96	0.47	2.57**	0.71
20:4 (n—6)	9.30	1.72	7.27*	1.32	8.70*	2.11	9.23*	1.38	8.25	2.18	8.88**	0.66
22:4 (n—6)	0.24	0.06	0.56	0.37	0.30	0.28	0.85	1.85	0.14	0.15	0.03	0.04
22:5 (n—6)	0.11	0.05	0.03	0.04	0.09	0.02	0.08	0.08	0.08	0.10	—	
22:5 (n—3)	0.82	0.29	0.32	0.25	0.59	0.25	0.39	0.26	0.55	0.04	0.56	0.43
22:6 (n—3)	0.24	0.76	1.55*	0.71	2.15*	0.98	1.69*	0.75	2.25*	0.73	2.51**	1.08

*Significant on 0.05 level (Bartlett's test): non-prednisone-treated against prednisone-treated.
**Significant on 0.05 level: blood donors against non-prednisone-treated progressive polyarthritics.

It seems that corticosteroids have some influence on metabolism of polyunsaturated fatty acids. Therefore it is necessary during studies of fatty acid profiles in multiple sclerosis patients to separate those who are under treatment with corticosteroids and those who are not. A detailed control study with a monitored lipid diet is necessary.

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