ARACHIDONIC ACID METABOLISM IN PLATELETS OF PATIENTS WITH FAMILIAL MEDITERRANEAN FEVER

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Familial Mediterranean fever (FMF) is a genetically determined disease characterized by syndromes of pain and fever, and based on inflammation of the serous membranes. Frequently it leads to severe amyloidosis of the kidneys, and the disease is considered at present to be virtually incurable.

Although the pathogenesis of the disease has not been adequately studied, it is classed as a collagenosis, for the treatment of which hyperbaric oxygenation (HBO) has been used with some success [2, 3]. The present writers have shown [1] that this treatment is also effective in FMF.

Meanwhile patients with FMF have been shown to have reduced aggregating ability of their platelets when stimulated by collagen [1], which is in accordance with the hypothesis that the pathogenesis of FMF is based on a deficiency of thromboxane synthetase [9], an enzyme participating in biosynthesis of thromboxane A_2 (TxA_2) [6]. TxA_2 is one of the most important of the autacoids of the icosanoid family, inducing, in particular, platelet aggregation [6]. This hypothesis is based on the fact that the attacks in patients with FMS are alleviated by treatment with colchicine, which, as we know, stimulates TxA_2 biosynthesis, and also with nonsteroid anti-inflammatory agents, such as inhibitors of postaglandin biosynthesis, which cause pain and fever. TxA_2 in turn inhibits prostaglandin (PG) biosynthesis by a feedback mechanism [9].

In the investigation described below the ability of platelets from patients with FMF to metabolize arachidonic acid (AA) into TxA_2, 12(S)-12-hydroxy-5Z,8Z,10E,14Z-icosatraenoic acid (12-HETE), and 12(S)-12-hydroxy-5Z,8Z,10E-heptadecatrienoic acid (12-HHT) — the principal metabolites of AA in human platelets [5, 6] — was studied. Incidentally, changes in biosynthesis of TxA_2 and other metabolites of AA in platelets, such as 12-HETE, may take place in association with widely different microvascular and immunologic disturbances in the body [4, 9], characteristic of the collagenoses [7, 9] and of FMF in particular.

EXPERIMENTAL METHOD

Blood (9 ml) from patients with FMF and from healthy blood donors was collected in polyethylene vessels with a 3.8% solution of sodium citrate (9:1 by volume) and centrifuged for 15 min at 200g. The top layer was withdrawn and centrifuged at 650g for 20 min, whereas the residue was resuspended in 10 ml of 0.15 M NaCl, 0.15 M Tris-HCl, 0.077 M EDTA (90:8:2), pH 7.4, and again centrifuged for 15 min at 650g. The residue was resuspended in 1 ml of buffer solution — 0.15M NaCl, 0.015 M Tris-HCl, 0.005 M glucose, pH 7.4 — and an aliquot was taken for platelet counting (the picoscale microparticle counter), after which their number was adjusted to $10^6/\mu l$. To 500 µl of the platelet suspension at 37°C 1 µl of a standard solution of 1-^{14}C-AA was added (0.1 µCi, 60 µCi/µmole, Amersham International, England). The final concentration of the solution was 66 µM. The mixture was incubated for 3 min and the reaction stopped by addition of 0.5 ml of 1 N HCl and the product extracted with 10 ml chloroform. The solvent was removed by evaporation and the residue applied to silica-gel plates (Merck, West Germany). Chromatography was carried out in a system of

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TABLE 1, Content (in %) of 1-^{14}C-AA and Its Metabolites in Incubation Medium with Platelets from Healthy Bood Donors and Patients with FMF 3 min after Beginning of Incubation (M ± m)

Substance	Healthy blood donors (n = 14)	Patients with FMF (n = 19)
AA	32,9+5,6	90,6+2,3
12-HETE	26,7+4,0	2,6+0,9
12-HHT	14,3+2,8	2,6+0,6
HEpA	14,1+2,8	—
TxB_2	9,2+1,8	2,3+0,6

Note. The rather high figure given for HEpA (10-hydroxy-11,12-epoxyicosatrienoic acid) may perhaps be the result of non-zymic conversion of 12-HPETE under the influence of 1-N HCl [13, 15].

solvents consisting of chloroform—methanol—acetic acid—water (90:8:1:0.8). The plates were sprinkled with a 14% chloroform solution of PPO and exposed with Orwo x-ray film for 1 week. After development of the autoradiograph the radioactive zones of silica-gel were scraped out and radioactivity of the samples was measured with a β-scintillation spectrometer (Roche Bioélectronique, France), using Bray's mixture as the scintillation fluid. The results were subjected to statistical analysis by the Fisher—Student test. Blood from 26 patients with FMF and 14 healthy blood donors was used.

Sessions of HBO were given in therapeutic pressure chambers of the OKA-MT type, for a toal duration of 45-50 min, with the following program: compression 0.05 atm, 5 min; exposure 1.2-1.3 atm, 40 min; decompression 0.05 atm, 5 min.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that on incubation of AA with platelets from patients with FMF, formation of TxA_2, 12-HETE, and 12-HHT took place to a much lesser degree than on incubation with platelets from the healthy blood donors. Whereas in the blood donors about 70% of AA underwent enzymic oxidation, in patients with FMF only 10% of AA was converted into TxA_2, 12-HETE, and 12-HHT.

It can be concluded on the basis of these results that in patients with FMF, activity of 12-lipoxygenase and cyclo-oxygenase in the platelets is inhibited, or these enzymes are deficient. Cyclo-oxygenase activity can be judged by the amount of 12-HHT formed from the TxA_2 precursor — PGG_2 cyclic endoperoxide [5].

The next step in the investigations was to study AA metabolism in human platelets during an attack, and also after a session of HBO, as a result of which the patients' pain was relieved and their subjective state improved. Since oxygen is known to stimulate TxA_2 biosynthesis selectively and to inhibit biosynthesis of other PG [9], it might be expected that during HBO TxA_2 biosynthesis would be intensified and biosynthesis of other PG would be reduced. Activation of free-radical oxiation of lipids leads to destruction of cyclo-oxygenase, which also causes inhibition of PG biosynthesis [8].

During the period of an attack and immediately after a session of HBO seven patients were investigated. However, no appreciable changes in lipo-oxygenase, cyclo-oxygenase, and thromboxane synthetase activity could be discovered. This fact is evidence that although TxA_2 biosynthesis is depressed in patients with FMF, attacks of pain and fever, inflammation of the serous membrane, and changes in ability of the patients' platelets to aggregate are linked with other highly active modulators of inflammation and anaphylaxis, and perhaps with leukotrienes [11], lipoxins [10], oxy- and hydroperoxy-acids (12-HETE, 5-HETE, 12-HPETE, 15-HPETE, etc.) [4], and with platelet activating factor (PAF) [14]. This is shown by the fact that in patients with FMF we found a very low level of 12-HETE synthesis, i.e., activity of platelet lipo-oxygenase was sharply inhibited, but 12-HETE and 12-HPETE in turn influence 5-lipo-oxygenase activity and leukotriene biosynthesis [12]. Taking into account also reports of selective inhibition of biosynthesis of leukotriene B_4 (a stimulator of hemotaxis of leukocytes, of secretion of lysosomal enzymes and of the superoxide anion) by colchicine

[11], the participation of AA metabolites in the pathogenesis of FMF becomes more evident still.

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DETECTION OF GLUCOCORTICOID-SENSITIVE DNA SEQUENCES IN AKR MOUSE THYMUS AND THEIR INACTIVATION IN THYMOMA

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Glucocorticoids are known to affect differentiation and functional activity of cell populations of the immune system. The discovery of glucocorticoid receptor complexes in nuclei of thymocytes and spleen cells [15] is evidence that glucocorticoids act on lymphoid cells by a mechanism of genetic induction: binding of glucocorticoid receptors complexes with hormone-sensitive regions of chromatin induces synthesis of specific mRNA [12]. It was shown previously that cortisol induction causes an increase in the content of moderately repeated sequences [2], including, it is considered, regulatory regions of the genes controlled by glucocorticoids [8], in the transcriptionally active DNA fraction (TA DNA) of rat liver. It was logical to suggest that the action of glucocorticoids in lymphoid tissue, just as in the liver, takes place through activation of regulatory regions in the genome. It also seemed probable that disturbances of mechanisms of regulation in transformed lymphocytes (for example, in lymphatic leukemia) may be the result of conformational changes in chromatin in the region of repeat sequences (RS) of DNA.

The aim of this investigation was to compare the distribution of glucocorticoid-controlled RS between functionally different DNA fractions in AKR mouse thymoma and also in the

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