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PROTEIN, RNA, AND DNA SYNTHESIS IN CULTURES OF SKIN FIBROBLASTS FROM HEALTHY SUBJECTS AND PATIENTS WITH RHEUMATIC DISEASES

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UDC 616-002.77-07:616.5-008.939.6-074

KEY WORDS: DNA; RNA; protein; skin fibroblasts; rheumatoid arthritis, systemic scleroderma.

Skin fibroblasts from patients with rheumatic fever, rheumatoid arthritis (RA) and systemic scleroderma (SS) possess enhanced functional activity, manifested as increased production of collagen, proteoglycans, and glycoproteins [1, 2, 4, 5]. A considerable increase in the rate of collagen biosynthesis by fibroblasts persisted during long-term cell culture, indicating a lasting disturbance of regulation of this process, possibly at the genome level, in patients with SS [5].

To study the mechanism of the lasting disturbance of fibroblast function, protein, RNA, and DNA synthesis was investigated in skin fibroblasts from patients with RA and SS.

EXPERIMENTAL METHOD

Strains of skin fibroblasts grown in the Laboratory of Biochemistry of Connective Tissue, Institute of Rheumatology, Academy of Medical Sciences of the USSR, were used. Cultures were tested at the third and fourth passages in the stationary phase of growth. The culture medium contained 50% of Eagle's medium, 30% of albumin hydrolysate, and 20% of bovine serum. The labeled precursors used to analyze synthesis of protein, RNA, and DNA were ^{14}C-protein hydrolysate (Czechoslovakia), [^{14}C]uridine (Czechoslovakia), and [^{14}C]thymidine (USSR) respectively. The labeling time was 4 h for the study of protein synthesis, and 2 h for the study of RNA and DNA synthesis. To investigate the stimulating effect of bovine embryonic serum (from the N. F. Gamaleya Institute of Microbiology and Epidemiology, Academy of Medical Sciences of the USSR) 5% serum was added to Eagle's medium and stimulation was determined by measuring incorporation of [^{14}C]proline (Czechoslovakia) into fibroblast proteins. The labeling time was 2 h. When actinomycin D (from "Reanal," Hungary) was used in these experiments it was either preincubated with the cells for 1 h or added to the Eagle's medium along with the [^{14}C]proline. To determine the rate of fission of prelabeled RNA after incubation of the fibroblasts for 1 h with [^{14}C]uridine, the cells were rinsed twice with Earle's medium and then incubated in growth medium (medium 199 with 20% bovine serum) with the addition of 50 μg/ml of uridine (from "Reanal"). All experiments with labeled precursors ended with a single washing of the cells with ice-cold Earle's medium.

During analysis of stability of fast-labeled RNA tests were carried out to discover whether all measurable radioactivity belonged to RNA molecules. For this purpose the fibroblasts were hydrolyzed in 0.3N KOH for 1 h at 37°C, after which they were precipitated with N $HClO_4$ and the precipitate applied to millipore filters (Synpor, No. 2, Czechoslovakia). The amount of radioactivity remaining in the cells was about 5% of its amount in unhydrolyzed

Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 2, pp. 156-158, February, 1985. Original article submitted June 15, 1984.

 0007-4888/85/9902-0180$09.50 © 1985 Plenum Publishing Corporation

TABLE 1. Protein, RNA, and DNA Synthesis in Skin Fibroblasts of Healthy Individuals and Patients with RA and SS

Strain of skin fibroblasts	Uptake, cpm/10^6 cells		
	of [^{14}C]uridine	of [^{14}C]thymidine	of ^{14}C-protein hydrolysate
Healthy individuals	23 908	871	8 642
	91 599	845	12 614
	88 525	730	4 223
	31 690	580	7 558
	58 930±18 055	757±66	8 259±1 729
Patients with RA	149 780	2 666	80 412
	347 126	6 255	219 798
	158 747	25 995	39 443
	128 745	12 800	31 886
	196 100±50 733	11 929±5 137	92 885±43 627
Patients with SS	86 283	1 167	52 735
	173 467	3 053	36 086
	150 333	6 460	47 166
	208 158	1 730	29 016
	53 670		158 254
	69 830		19 324
	123 624±25 507	3 102±1 187	57 097±20 818

TABLE 2. Stability of Fast-Labeled RNA in Skin Fibroblasts from Healthy Individuals and Patients with RA and SS (mean results of three experiments)

Strain of skin fibroblasts	Labeling for 1h	Time after rinsing to remove [^{14}C]uridine, h			
		1	2	3	4
		cpm/10^6 cells			
Patients with RA	14 162	14 508 (102)	16 042 (113)	14 728 (104)	15 999 (113)
Healthy individuals	18 762	18 758 (100)	13 758 (73)	13 319 (71)	13 519 (72)
Patients with SS	69 830	52 712 (75)	43 977 (63)	41 769 (60)	

Legend. Percentage of residual prelabeled RNA shown in parentheses.

cells. Lysis of the cells was carried out, in the case of investigation of protein synthesis, by a solution containing 0.4% sodium deoxycholate in 0.1 N KOH [8], and for investigation of RNA and DNA synthesis, with a solution containing 0.001 M Tris-HCl, pH 7.5, 0.005 M EDTA, and 0.2% sodium dodecylsulfate [7]. Acid-insoluble material was precipitated by 10% TCA solution and growth medium (0.1 ml) was used as the carrier. Radioactivity of the samples was determined with a Mark II (Nuclear Chicago, USA) counter in a toluene–POPOP system. Cells were counted in a Goryaev's chamber.

EXPERIMENTAL RESULTS

Protein synthesis in skin fibroblasts from patients with SS was at a rate almost six times higher than in skin fibroblasts of healthy individuals and in skin fibroblasts from patients with RA it was 11 times higher than normal (Table 1). DNA synthesis in skin fibroblasts of patients with RA was 15 times higher, and in skin fibroblasts from patients with SS four times higher than normal. The level of synthesis of fast-labeled (chiefly mRNA) RNA was about two or three times higher than normal in both diseases. The results indicate a higher level of DNA synthesis and higher activity of the protein-synthesizing system in skin fibroblasts of patients with RA.

TABLE 3. Effect of Embryonic Calf Serum and Actinomycin D on [^{14}C]Proline Uptake (in cpm/10^6 cells) into Proteins of Skin Fibroblasts from Healthy Individuals and Patients with RA and SS

Strain of skin fibroblasts	Effect on uptake [^{14}C]proline, %	
	of 5% embryonic calf serum*	of actinomycin D**
Healthy individuals	154	74
	243	58
	63	
	87	
	137±40	
Patients with RA	97	55
	81	29
	97	
	84	
	110	
	108	
	96±7	
Patients with SS	136	114
	209	96
	228	93
	434	92
	222	119
	355	103±6
	161	
	155	
	238±37	

Legend. *) Change in uptake, in percent, compared with Eagle's medium without serum; **) change in uptake, in percent, compared with medium (Eagle's medium with 5% embryonic calf serum), not containing actinomycin D.

Analysis of breakdown of fast-labeled RNA (Table 2) showed that virtually all RNA in skin fibroblasts from patients with RA consists of long-living RNA molecules, whereas in skin fibroblasts from patients with SS about 30% of the fast-labeled RNA breaks down in the course of 1-2 h.

The different levels of incorporation of [^{14}C]uridine into RNA (Tables 1 and 2) are connected with different experimental conditions (cells were incubated with [^{14}C]uridine for different times and different culture media were used).

Addition of 5% embryonic calf serum to Eagle's medium stimulated uptake of [^{14}C]proline by 16% in normal fibroblasts and about doubled it in skin fibroblasts from patients with SS (Table 3). If the fibroblasts were preincubated with actinomycin D the effect of stimulation of protein synthesis was abolished in normal fibroblasts only. The results may perhaps be linked with poor permeability of skin fibroblasts from patients with SS for actinomycin D. Indirect evidence in support of this view was obtained by the writers when studying the effect of actinomycin D on RNA synthesis in test fibroblasts. Actinomycin D in a dose of 1.8 μg/ml medium was found to inhibit RNA synthesis in skin fibroblasts from patients with SS by only 53-72%, whereas in skin fibroblasts from healthy individuals and from patients with RA actinomycin D in a dose of 0.1-0.2 μg/ml inhibited RNA synthesis by 86-97%.

With respect to the parameters chosen for study, skin fibroblasts from patients with RA and SS thus differ not only from normal fibroblasts, but also from each other, and this conclusion does not contradict data published previously [3, 6, 9-11].

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SOME KINETIC PROPERTIES OF PLASMA LECITHIN-CHOLESTEROL ACYLTRANSFERASE IN HYPER-α-LIPOPROTEINEMIA IN MAN

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UDC 616.153.963.915-008.61-07:[616.153.963.21+616.153.922+616.153.1:577.152.2

KEY WORDS: lecithin-cholesterol acyltransferase; high-density lipoproteins; hyper-α-lipoproteinemia.

Lecithin-cholesterol acyltransferase (LCAT; E.C. 2.3.1.43) catalyzes the transacylation reaction, namely transfer of a fatty acid radical from the β-position of lecithin to the hydroxyl group of cholesterol (CH) with the formation of a cholesterol ester (CHE) and lysolecithin [7, 8]. Both substrates (lecithin and cholesterol), and also apoproteins A-I and C-I which are activators of this reaction, are components of high-density lipoproteins (HDL), with which LCAT forms a substrate-enzyme complex in the blood [4]. Recently the protective role of HDL in the development of atherosclerosis and one of the complications of this disease, namely ischemic heart disease, has been demonstrated [2, 3, 12, 13, 15]. The biochemical mechanisms which lie at the basis of elevation of the blood HDL level and also the role of LCAT in this process remain unexplained. Albers [5] showed by analytical isofocusing that five LCAT isozymes are present in human blood plasma. These findings have not yet been confirmed. The physiological role of the individual isozymes likewise awaits elucidation.

The aim of this investigation was to study some kinetic properties of LCAT in the blood plasma of patients with hyper-α-lipoproteinemia, enabling the presence of LCAT isozymes in the blood to be detected.

EXPERIMENTAL METHOD

Blood was taken from healthy men aged 25-35 years with persistent hyper-α-lipoproteinemia (their HDL cholesterol, or α-CH level in all cases exceeded 80 mg/dl, corresponding to an HDL concentration of more than 500 mg/dl according to the results of analytical ultracentrifugation [1]). The total HDL fraction isolated from the subjects' blood plasma within the density range of 1.063-1.21 g/ml was used as substrate for the LCAT reaction. The source of enzyme was lipoprotein-free plasma obtained from the subjects of the investigation by removal of all classes of lipoproteins (LP) from it. The method of taking blood, obtaining plasma, isolating HDL, and obtaining 4-^{14}C-cholesterol-labeled HDL and lipoprotein-free

Department of Biochemistry, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 2, pp. 158-161, February, 1985. Original article submitted March 29, 1984.

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