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# CHANGES IN THE FATTY ACID COMPOSITION AND LIPID PEROXIDATION ACTIVITY OF LIVER MICROSOMAL MEMBRANES IN EXPERIMENTAL MYOCARDIAL INFARCTION

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KEY WORDS: myocardial infarction; liver microsomes; fatty acids; lipid peroxidation

The writers showed previously in acute myocardial infarction there is a long-lasting (for 3 weeks) decrease in hydroxylating activity and the quantitative content of enzymes of the liver mono-oxygenase system [5]. The mechanisms of inhibition of microsomal liver mono-oxygenation in myocardial infarction have not been studied.

The aim of this investigation was to study changes in the fatty-acid composition, hydrophobic properties, and activity of lipid peroxidation (LPO) of liver microsomal membranes in the course of acute coronary-occlusive myocardial infarction.

## EXPERIMENTAL METHOD

Experiments were carried out on 100 male Wistar rats weighing 180-220 g. In the experiments of series I, after left-sided thoracotomy and under ether anesthesia the left descending coronary artery of the animals was ligated. In the experiments of series II thoracotomy was performed but without ligation of the coronary artery (mock operation). Intact rats, kept under standard animal house conditions, served as the control. The animals were decapitated, 1, 3, 7, 14, and 21 days after the operation and the microsomal fraction of the liver was isolated [1]. Microsomal lipids were extracted by the method in [8]. The concentration of fatty acids in the lipid fraction were determined by gas chromatography on the "Chrom-4" instrument (Czechoslovakia), as described by the writers previously [7]. The resistance of the microsomal membranes to injury in vitro in NADPH-dependent LPO reactions was studied on the 6th, 14th, and 21st days of myocardial infarction [7]. The original parameters and their values after 10 min of induction of NADPH-dependent LPO were estimated: the rate of malonic dialdehyde (MDA) formation [2] and the cytochrome *P*-450 concentration [11]. Changes in hydrophobicity of the microsomal membranes were studied with the aid of the fluorescent probe 1-anilinenaphthalene-8-sulfonate (1,8-ANS$^-$) at the above-mentioned periods of infarction. The binding constant ($K_b$) for 1,8-ANS$^-$ was calculated by the method of double reciprocals [3, 7]. The probe was prepared in 40 mM Tris-HCl buffer (pH 7.4). The membranes (concentration 0.5 mg/ml) were titrated with the probe in the concentration range 5-40 $\mu$M. Fluorescence of the bound 1,8-ANS$^-$ was recorded on an MPF-4 fluorescence spectrophotometer (Hitachi, Japan). The wavelength of excitation was 360 nm and of fluorescence 480 nm. Spectral constants of interaction of a type II substrate (aniline) with cytochrome *P*-450 also were studied [4]. The spectral dissociation constant ($K_s$) for aniline and the maximal binding of aniline with cytochrome *P*-450 ($\Delta A_{max}$) were determined. The concentration of cytochrome *P*-450 and the differential spectra of binding of aniline with cytochrome were recorded on a Hitachi-356 spectrophotometer (Japan). The pro-

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TABLE 1. Fatty Acid Composition of Microsomal Lipids of Liver Membranes at Different Stages of Acute Coronary Occlusive Myocardial Infarction ($M \pm m$, $n$ = 6-10)

| Fatty acid, % | Exptl. conditions | Time after ligation of coronary artery, days | | | | | Control |
|---|---|---|---|---|---|---|---|
| | | 1st | 3rd | 7th | 14th | 21 st | |
| Myristic $C_{14:0}$ | I | 0,16±0,02 | 0,12±0,02 | 0,11±0,02 | 0,30±0,05* | 0,32±0,02* | 0,11±0,02 |
| | II | 0,07±0,02 | — | 0,05±0,01* | — | 0,25±0,03* | |
| Pentadecanic $C_{15:0}$ | I | 0,09±0,01 | 0,07±0,01 | 0,10±0,02 | 0,14±0,03* | 0,58±0,03* | 0,07±0,005 |
| | II | 0,06±0,01 | — | 0,03±0,01* | — | 0,28±0,04* | |
| Palmitic $C_{16:0}$ | I | 16,33±1,26 | 16,60±0,46 | 19,60±1,39 | 19,55±1,71 | 25,59±0,87* | 18,11±0,89 |
| | II | 14,79±0,92* | — | 10,42±0,49* | — | 15,02±1,33 | |
| Palmitoleic $C_{16:1}$ | I | 1,54±0,08 | 1,89±0,18 | 3,32±0,42* | 2,87±0,86 | 4,24±0,48* | 1,75±0,16 |
| | II | 1,38±0,08 | — | 0,86±0,08* | — | 3,63±1,03 | |
| Stearic $C_{18:0}$ | I | 25,37±1,49 | 26,0±0,67 | 22,38±0,31 | 20,79±1,49 | 18,77±0,80* | 23,59±0,91 |
| | II | 27,20±1,40* | — | 20,81±1,81 | — | 18,65±1,14* | |
| Oleic $C_{18:1}$ | I | 7,49±0,55* | 8,77±0,70 | 10,83±0,22 | 17,48±1,75* | 16,39±1,35* | 9,61±0,73 |
| | II | 10,06±0,43 | — | 9,86±0,90 | — | 15,82±0,71* | |
| Linoleic $C_{18:2}$ | I | 17,29±0,62 | 17,28±0,25 | 21,06±0,57* | 16,98±1,15 | 9,23±0,33* | 16,97±0,12 |
| | II | 16,73±0,99 | — | 18,25±0,68 | — | 15,17±0,88 | |
| Arachidonic $C_{20:4}$ | I | 31,22±1,69 | 29,04±0,82 | 21,96±1,73* | 21,14±1,69* | 19,44±1,48* | 29,21±1,06 |
| | II | 29,45±0,95 | — | 38,90±0,99* | — | 29,57±1,26 | |

**Legend.** Here and in Tables 2 and 3, asterisk indicates significance of differences compared with control ($p < 0.05$). I) Myocardial infarction; II) mock operation.

TABLE 2. Changes in Binding Parameters of 1,8-ANS⁻ and Aniline by Microsomal Fraction of the Liver during Experimental Myocardial Infarction ($M \pm m$, $n$ = 6)

| Parameter | Postinfarction period, days | | | Control |
|---|---|---|---|---|
| | 7-e | 14-e | 21-e | |
| $K_b$, μM | 18,08±1,56* | 13,51±0,53* | 17,36±1,03* | 12,04±0,48 |
| $K_s$, μM | 4,26±0,25* | 6,53±0,21* | 4,63±0,19* | 2,84±0,14 |
| $\Delta A_{max}$ | 9,39±0,39* | 8,22±0,88* | 10,78±0,91 | 11,41±0,35 |

$\Delta OD_{430-500} \times 10^3$/mg microsomal protein

**Legend.** OD) Optical density at corresponding wavelengths.

tein concentration in the microsomes was determined by the method in [10]. All the experimental data were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

The investigation showed that changes in the fatty acid composition of microsomal membrane lipids of the liver were most marked in the late stages (7th-21st day) of myocardial infarction. During this period the content of saturated fatty acids (myristic, pentadecanic, palmitic) and also of unsaturated palmitoleic acid was increased. On the 7th, 14th, and 21st days of infarction, a persistent fall of the arachidonic acid concentration was observed in the microsomal lipids. Changes in the linoleic acid concentration were biphasic in character: it rose on the 7th day, returned to the control level on the 14th day, and was below the control level on the 21st day. Changes in the spectrum of individual fatty acids on the 21st day of myocardial infarction led to an increase in the total content of saturated (46.2 ± 1.3) and a decrease in the total unsaturated (53.8 ± 1.3) fatty acids compared with the control (41.8 ± 0.74 and 58.1 ± 0.74, respectively, $p < 0.05$). Opposite changes in the spectrum of fatty acids in the microsomal lipids were observed in animals undergoing the mock operation. On the 7th day, the arachidonic acid concentration was increased but the palmitic acid concentration was significantly lowered, although by the 21st day their relative percentages did not differ from the control. The total content of saturated fatty acids on the 7th and 21st days was significantly lower, and that of the unsaturated acids higher, than the control (Table 1).

TABLE 3. Resistance of Microsomal Membranes of the Liver to Injury In Vitro during Induction of NADPH-Dependent LPO during Acute Occlusive Myocardial Infarction ($M \pm m$, $n = 6$)

| Time after ligation of coronary artery, days | Original value | | After induction of NADPH-dependent LPO | | |
|---|---|---|---|---|---|
| | cytochrome P-450 | $MDA_1$ | cytochrome-P-450 | $MDA_2$ | per cent loss of cytochrome P-450 |
| 7 | 0,79±0,04* | 0,107±0,01* | 0,69±0,03 | 0,62±0,01* | 12,3±1,8* |
| 14 | 0,83±0,04* | 0,062±0,002* | 0,75±0,04* | 0,61±0,02* | 9,7±1,8* |
| 21 | 0,90±0,01* | 0,083±0,002 | 0,32±0,04* | 2,06±0,06* | 64,3±4,5* |
| Control | 0,96±0,02 | 0,074±0,004 | 0,66±0,01 | 0,77±0,01 | 31,5±1,4 |

**Legend.** Cytochrome *P*-450 concentrations given in mmoles/mg microsomal protein, $MDA_1$) in nanomoles/mg microsomal protein, $MDA_2$) in mmoles/min/mg protein; loss of cytochrome *P*-450 expressed as percentage of initial concentration, taken as 100%.

The main function of the microsomal lipids is known to be maintenance of hydrophobic conditions for catalytic processes [6]. The study of the parameters of binding of the hydrophobic fluorescent probe 1,8-ANS$^-$ showed that $K_b$ on the 7th, 14th, and 21st days of infarction, was increased for the probe by 50.2, 53.7, and 44.2%, respectively, evidence of a decrease of its affinity for the membrane. The increase in $K_s$ for aniline by 1.5, 2.4, and 1.6 times, respectively, indicates, in addition, a decrease in the affinity of cytochrome *P*-450 for type II substrates. On the 7th and 14th days, the concentration of "binding protein" in the microsomes also was reduced for type II substrates, as shown by reduction of $\Delta A_{max}$ for aniline at these times (Table 2).

One of the factors modulating the fatty acid composition of lipids is the activity of peroxidative processes [2]. The study of activity of spontaneous LPO, from this standpoint, showed that the MDA concentration on the 7th day of myocardial infarction was 44.6% higher than in the control. On the 14th day of infarction the MDA concentration was 16.2% below the control level, and it returned to its initial value on the 21st day. During induction of NADPH-dependent LPO the rate of MDA formation on the 7th and 14th days was depressed by 20.0 and 21.0%, respectively, but on the 21st day it was 2.7 times higher than in the control (Table 3).

The results suggest that the increase in spontaneous LPO activity observed on the 7th day of myocardial infarction leads to a decrease in the content of polyunsaturated fatty acids (PUFA), substrates for LPO, in the lipids of the microsomes [2]. As a result of the reorganization of the fatty acid composition, first, hydrophobicity of the microsomal membranes was reduced, as shown by changes in the parameters of binding of 1,8-ANS$^-$ [7, 9], and in the kinetics of enzyme–substrate interaction [5, 6]. Second, it led to an increase in the concentration of saturated fatty acids, which evidently gave rise to increased packing density of the membrane phospholipids [7, 9]. This "condensation" of the membranes is shown by an increase in their resistance to induction of NADPH-dependent LPO on the 7th and 14th days and prevention of considerable damage to cytochrome *P*-450 during these periods (Table 3). However, the period of increased resistance of microsomal membranes to injury in reactions of NADPH-dependent LPO was replaced on the 21st day of myocardial infarction by a considerable fall in this parameter. The transition of the membranes into a state of "relaxation" during this period can be explained by the disorderly arrangement of molecules of the membrane phospholipids as a result of a significant increase in the fraction of monounsaturated fatty acids in their composition. The biphasic character of changes in the structural organization of microsomal membranes also has been observed in ischemia of the liver [7].

Changes arising in the structural organization of microsomal membranes of the liver in the course of myocardial infarction must therefore be regarded as adaptive changes, preventing profound damage to membrane-bound enzymes. These changes are also the cause of limitation of functional activity of the liver mono-oxygenase system in the recovery period of myocardial infarction.

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# DYNAMICS OF ALKALINE HYDROLYSIS OF RNA OLIGOADENYLATES DURING HYDROLYSIS OF HOMOGENATES OF RAT CEREBRAL CORTEX AND LIVER

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Attention has been drawn in the literature to the exceptional resistance to alkaline hydrolysis of RNA fragments and of individual ribo-oligonucleotides, consisting of chains of adenyl nucleotides cross-linked by a 3′,5′-phosphodiester bond, namely 3′,5′-oligoadenylates (3′,5′-A), which is largely responsible for the effects of interplanar interaction between adenine bases [6]. Many such regions, consisting of 100-200 AMP molecules, also contain *m*RNAs and their precursors [10]. The functional role of the polyadenyl fragments of *m*RNA has not yet been finally settled, largely due to the absence of any simple methods of their quantitative determination [10].

It has been shown that 3′,5′-A can exhibit physiological activity similar to that of 2′,5′-oligoadenylates (2′,5′-A), which, together with *c*AMP, are mediators of the single regulatory system of the cells [2]. At the present time a search is being made among the 2′,5′-A for effective antiviral and antitumor therapeutic agents [4]. Considering also that 3′,5′-A can be converted into 2′,5′-A by the action of weakly basic ion-exchange resins [6], the results of the study of hydrolysis of RNA oligoadenylates during alkaline hydrolysis of tissue homogenates, which are not to be found in the literature, are not only of theoretical, but also of practical importance.

## EXPERIMENTAL METHOD

Alkaline hydrolysis products of RNA were obtained by the method of Schmidt and Thannhauser in Trudolyubova's modification [8]. Samples of tissue homogenates were incubated at 37°C for 1, 5, and 20 h in 0.3 M KOH [8]. After precipitation of DNA and proteins by perchloric acid, the resulting alkaline RNA digests were neutralized with ammonia and reprecipitated with $NH_4Cl$ saturated with isopropanol (1:20), and then filtered on four layers of "Filtrac-90" filter paper (East Germany), under a vacuum to remove the salts. After the filters had been washed with isopropanol and ether the alkaline hydrolysis products of RNA adsorbed on the paper were washed off with bidistilled water and evaporated to dryness in vacuo at 37°C. The

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