EDITORIAL

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The role of lipid peroxidation in pathogenesis of ischemic damage and the antioxidant protection of the heart

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Summary

A working hypothesis on pathogenesis of ischemic heart damage has been proposed. According to this hypothesis, a crucial role in conversion of reversible damage into irreversible damage is played by cardiomyocyte membrane destruction caused by the so-called "lipid triad". The latter comprises activation of lipid peroxidation, activation of phospholipases, and the detergentlike action of excessive amounts of free fatty acids and lysophospholipids. Marked activation of lipid peroxidation in experimental myocardial infarction, as well as reoxygenation following transitory ischemia, have been demonstrated. The proposed hypothesis and experimental data underly successful application of synthetic free radical scavengers (antioxidants) for heart protection against experimental myocardial infarction, transitory ischemia, and emotional, painful stress.

Key words: myocardial infarction, reoxygenation, stress, lipid peroxidation, free radical scavengers

Introduction

Lipid peroxidation (LPO) is a continuous physiological process occurring in cell membranes. Apart from being a membrane renewal factor, this process is an essential step in biosynthesis of prostaglandins and leukotrienes as well as in phagocytosis, pinocytosis, disassembly of intracellular membranes, etc. (6, 26, 27, 52, 53). Recent studies have demonstrated that excessive activation of LPO plays an important and sometimes a key role in the development of many diseases. Primarily the activation of LPO has been found to be involved in various damages induced by hyperbaric oxygenation (16, 22), X-radiation (10, 55), and deficiency of vitamin E, the predominant natural free radical scavenger (4, 13).

It has recently been reported that LPO activation inevitably occurs in the heart and other organs of animals exposed to stress (45); the stressory damages can be prevented by administration of natural and synthetic free radical scavengers (44, 46). Since the role of stressory situations in the etiology of the main endogenous noninfectious diseases is quite obvious, LPO activation appears to be a common pathogenetic mechanism of these disturbances (40). Indeed, there are some literature data on the important role of this process in the development of gastric mucosa ulceration (47), retinal damage (54), stressory damage of brain metabolism (7), neoplasms (28, 32), atherosclerosis (15, 58), and, finally, cardiac ischemia (9, 31, 43).

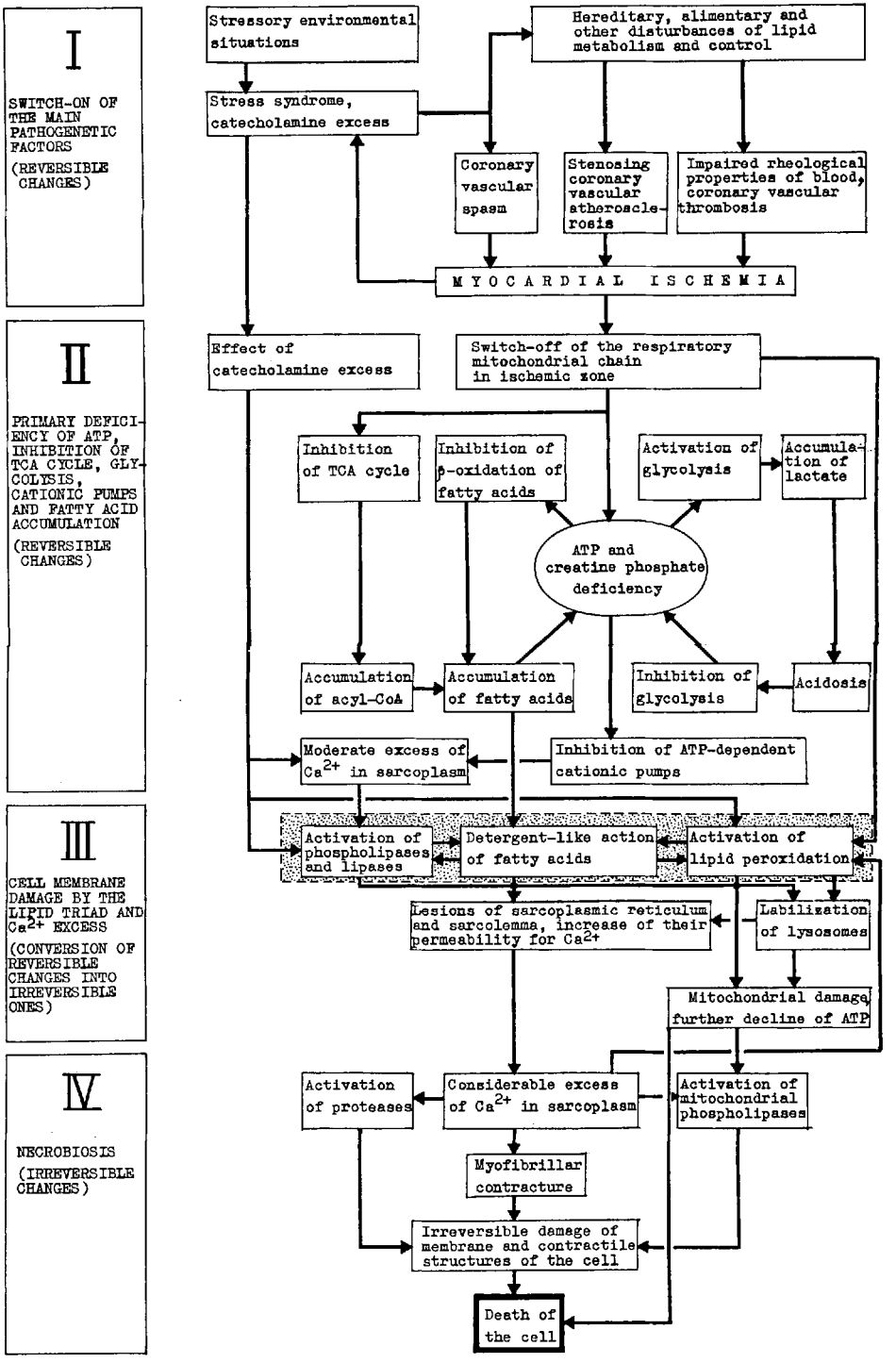
The aim of the present investigation was to study the role of LPO activation in pathogenesis of ischemic, hypoxic and reoxygenation damages of the heart and to evaluate the possibility of application of free radical scavengers for prevention of such damage. From this viewpoint we shall consider the three following points: i) present-day concepts on the pathogenesis of ischemic heart damage and the role of LPO in this process, ii) the nature of LPO, and iii) the use of synthetic free radical scavengers for prophylaxis of ischemic, hypoxic and reoxygenation damages of heart muscle.

Pathogenesis of ischemic heart damage and the role of LPO activation in this process

The concepts on pathogenesis of ischemic heart damage are far from being unambiguous and are rapidly changing. The scheme presented below is a working hypothesis which sums up the data obtained in the last few years (1–3, 49, 50). According to the authors of the present article, the formation of the pathogenetic chain of myocardial infarction comprises four steps (fig. 1).

The first step is a switch-on of the main pathogenetic factors. As can be seen in figure 1, the two etiological factors – environmental stressory situations, on the one hand, as well as hereditary and alimentary disorders of lipid metabolism and of its control, on the other hand, produce at least three primary shifts in the organism, namely stenosing coronary vascular atherosclerosis, stress syndrome, and, finally, changes in the rheological properties of the blood, and more or less pronounced coronary artery thrombosis. The main event constituting the essence of the disease, i.e., myocardial ischemia, is produced either indirectly by the stress syndrome (coronary vascular spasm) or directly by the other two above-mentioned shifts. Beside its main manifestation, switch-off of the mitochondrial respiratory chain, myocardial ischemia is inevitably associated with pain which, in turn, enhances or switches on the stress syndrome again and causes considerable stimulation of adrenergic regulation and catecholamine excess in the organism. In this connection the concept put forward by Professor E. I. Chazov's clinic that "infarction is always stress" seems to be most plausible. It should only be added that such stress is essentially

Fig. 1. A schematic representation of pathogenesis of myocardial infarction. For details see Text.


I
SWITCH-ON OF THE MAIN PATHOGENETIC FACTORS
(REVERSIBLE CHANGES)
II
PRIMARY DEFICIENCY OF ATP, INHIBITION OF TCA CYCLE, GLYCOLYSIS, CATIONIC PUMPS AND FATTY ACID ACCUMULATION
(REVERSIBLE CHANGES)
III
CELL MEMBRANE DAMAGE BY THE LIPID TRIAD AND Ca^{2+} EXCESS
(CONVERSION OF REVERSIBLE CHANGES INTO IRREVERSIBLE ONES)
IV
NECROBIOSIS
(IRREVERSIBLE CHANGES)
Stressory environmental situations
Hereditary, alimentary and other disturbances of lipid metabolism and control
Stress syndrome, catecholamine excess
Coronary vascular spasm
Stenosing coronary vascular atherosclerosis
Impaired rheological properties of blood, coronary vascular thrombosis
MYOCARDIAL ISCHEMIA
Effect of catecholamine excess
Switch-off of the respiratory mitochondrial chain in ischemic zone
Inhibition of TCA cycle
Inhibition of β-oxidation of fatty acids
Activation of glycolysis
Accumulation of lactate
ATP and creatine phosphate deficiency
Accumulation of acyl-CoA
Accumulation of fatty acids
Inhibition of glycolysis
Acidosis
Moderate excess of Ca^{2+} in sarcoplasm
Inhibition of ATP-dependent cationic pumps
Activation of phospholipases and lipases
Detergent-like action of fatty acids
Activation of lipid peroxidation
Lesions of sarcoplasmic reticulum and sarcolemma, increase of their permeability for Ca^{2+}
Labilization of lysosomes
Mitochondrial damage, further decline of ATP
Activation of proteases
Considerable excess of Ca^{2+} in sarcoplasm
Activation of mitochondrial phospholipases
Myofibrillar contracture
Irreversible damage of membrane and contractile structures of the cell
Death of the cell

emotional, painful stress, i.e., it is caused by pain and by fear of death (11, 51). The proposed scheme demonstrates that this stage results in two events, namely, switch-off of the mitochondrial respiratory chain in the ischemic zone of the myocardium and appearance of catecholamine excess, which is caused by the stress syndrome.

These two events give rise to a second step consisting in primary deficiency of ATP, inhibition of the tricarboxylic acid cycle, glycolysis, ATP-dependent cation pumps and fatty acid accumulation. Fig. 1 shows that the switch-off of the respiratory chain at this stage directly causes the inhibition of the tricarboxylic acid cycle and deficiency of energy-rich phosphorous compounds. The latter two shifts induce activation and subsequent inhibition of glycolysis and fatty acid accumulation via a well-established sequence of reactions. This process is paralleled by simultaneous inhibition of the slow channel of electrogenic Ca^{2+} influx into cardiomyocytes and by instantaneous depression of heart contractility, which is followed by inhibition of the ATP-dependent cation pumps and, consequently, initial moderate excess of Ca^{2+} in the sarcoplasm.

All these shifts appear to be reversible; they produce, however, essential changes in the lipid bilayer of the myocardial cells. These changes, which may be termed as "the lipid triad of membrane damage", form a basis for the third step shown in the scheme, i.e., conversion of reversible myocardial lesions to irreversible lesions. The lipid triad is essentially potentiated by catecholamine excess, and consists in activation of lipases and phospholipases, detergentlike action of fatty acids and lysophospholipids on the membranes, and, finally, activation of LPO. When evaluating the triad components, one should consider two facts. Firstly, the activation of LPO and phospholipases occurs in cardiomyocytes, while the effects of the third component of the triad are brought about by catecholamine-induced activation of lipases in adipose and other extracardiac tissues. The resulting increase of fatty acid content in the blood under infarction and stress is an indirect cause of membrane damage. Secondly, in contrast to phospholipid hydrolysis LPO is an irreversible process, since the cell is devoid of mechanisms capable of utilizing LPO end products. It can also be seen from the scheme that the lipid triad brings about labilization of lysosomes; as well as lesions of sarcolemma, sarcoplasmic reticulum and mitochondria; and causes increased permeability of these membrane structures for Ca^{2+}. Apparently, the reversibility or irreversibility of these damages depends on the degree of their severity.

In prolonged ischemia, the structural membrane damages are aggravated and constitute a prerequisite for the fourth step of the process, i.e., occurrence of necrobiosis. The scheme shows that the excess of Ca^{2+} accumulated as a result of structural damage of sarcolemma and other membrane structures of the cell causes contracture of myofibrils and their destruction by myofibrillar proteases; this additional activation of the lipid triad thus stimulates membrane destruction. The membrane destruction eventually results in irreversible damage of all the main organelles of cardiomyocytes and in death of these cells, which underlies irreversible cessation of the contractile function of ischemic heart muscle and myocardial infarction.

One of the consequences of the above considerations consists in the following. Properly timed inhibition of the lipid triad, in particular, LPO, phospholipases and lipases, can reduce membrane damage and thus prevent the conversion of reversible ischemic damage to irreversible damage. In clinical cardiology the significance of LPO and inhibition of LPO by free radical scavengers should therefore be evaluated from this viewpoint.

Mechanisms of activation and damaging effect of lipid peroxidation

When considering the problem of the nature of LPO, one should consider that oxygen utilization in the organism occurs via two pathways. The first one, the oxidase pathway, has been widely investigated and consists in oxidation of energy substrates by the terminal component of the respiratory chain, cytochrome oxidase. As a result of this process, oxygen accepts four electrons and is reduced to form H_2O. The oxidase pathway does not involve O_2 incorporation into the oxidizing substrate molecule; under normal conditions it is coupled with ATP synthesis, thus serving as an energy source for living systems. Therefore, this pathway has always attracted the attention of biologists and, particularly, of cardiologists.

The second, much less investigated pathway, the oxygenase pathway, does not involve complete four-electron reduction of O_2, but consists in a stepwise reduction and formation of activated oxygen species via acceptance of one, two or three electrons by O_2. The respective products are superoxide anion radicals, hydrogen peroxide and hydroxyl radicals. These activated oxygen species can interact with endogenous substrates in the cell; this process is accompanied by incorporation of one atom or of the whole molecule into the oxidizing substrate. Endogenous lipids play an essential role in free radical oxidation. The free radical oxidation of the lipids gives rise to peroxyderivatives; therefore the whole process has been termed LPO.

In order to understand the mechanism of superfluous damaging activation of LPO, one should consider that under certain conditions the process of oxygen utilization can be switched from the oxidase (respiratory) pathway to the oxygenase pathway; the latter forms a basis for LPO. Indeed, the four-electron reduction of O_2 to H_2O in cytochrome oxidase only succeeds with continuous and well-coordinated functioning of the whole ensemble of the respiratory chain carriers. When the terminal component of the respiratory chain is inhibited (e.g., in ischemia, anoxia), the inevitable reduction of NAD to NADH and that of respiratory chain carriers may eventually result in reduction of molecular oxygen dissolved in the lipid matrix of the membrane. However, this reduction will be other than a four-electron, complete reduction of O_2 to H_2O; it will be incomplete reduction coupled with the formation of activated oxygen species. Of course, the production of such species is only possible when ischemia is not associated with exhaustive anoxia and when the lipid matrix contains sufficient amounts of O_2.

It seems essential to point out that enhanced production of free radicals in the whole organism can occur not only in ischemia or anoxia, but also in an entirely different situation as well, namely, under hyperbaric oxygena-

tion. In such a situation the amount of O_2 dissolved in the membrane lipid matrix is sharply elevated and the possibility of O_2 interaction with the reduced electron carriers in mitochondrial and microsomal electron transporting chains is correspondingly increased despite the small number of carriers.

Presumably the strongest activation of the LPO process could be expected when both of the above conditions occur simultaneously, that is, when prolonged anoxia and a considerable accumulation of reduced carriers are followed by excessive supply of O_2, i.e., in reoxygenation preceded by prolonged hypoxia or ischemia. Hence, LPO activation can result from two oppositely directed changes in O_2 balance in the cell. In ischemia or anoxia LPO activation is due to the excess of electron donors, i.e., reduced carriers, while in hyperbaric oxygenation LPO activation is due to the excess of the electron acceptor, i.e., molecular O_2. And, finally, during reoxygenation LPO activation is a result of simultaneous action of these two factors. It is noteworthy that the conditions of postischemic or posthypoxic reoxygenation (which is presently termed "oxygen paradox") have been studied in great detail. It has also been shown that these reoxygenation situations are associated with intensive accumulation of LPO products (17, 18) which cause severe damage to cell structures of the heart and other organs (20, 21).

In our opinion, this observation of marked accumulation of LPO products is very important for cardiological practice since reoxygenation is a common phenomenon in the course of development of ischemic disease. Reoxygenation is inevitably concomitant with the final stage of a stenocardiac attack resulting from coronary vasospasm, and with reactive hyperthermia, collateral opening, or natural reperfusion under conditions of coronary artery occlusion.

The scheme shown in figure 2 illustrates the formation of primary molecular products of LPO (phospholipid hydroperoxides) which occurs as a result of incorporation of the O_2 molecule into unsaturated fatty acid residues. The thus-formed hydroperoxides are unstable and are readily decomposed to form a large number of secondary and end products of

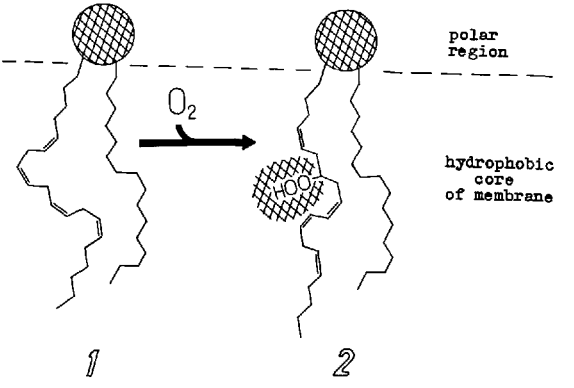


Fig. 2. Interaction of phospholipid (1) with O_2 and formation of phospholipid hydroperoxide (2).

LPO. The effects of LPO on biomembranes can be classified in at least four groups: a) changes in the lipid microenvironment of membrane-bound enzymes, receptors and ion channel formers, which can both activate and inhibit the functional activity of such proteins; b) formation of new permeability channels (see below); c) formation of cross-links between proteins and phospholipids of biomembranes, coupled with irreversible inactivation of the protein and; d) oxidation of SH-groups in the active sites of membrane-bound enzymes, and a resulting loss of enzyme functional properties (5, 24, 33–35, 41, 48). One of these effects, the formation of peroxide clusters, is presented schematically in figure 3. It can be seen that as phospholipid hydroperoxides are accumulated in the membrane, their lateral diffusion first results in their association in each monolayer, and subsequently in the formation of transmembrane peroxy clusters; the clusters serve as ion permeability channels, particularly for Ca^{2+} (25, 35, 38, 48). It may be assumed that the formation of such clusters during LPO activation induced by ischemia, stress or other disorders can play an essential role in Ca^{2+} excess accumulation in myocytes and in manifestation of the damaging effect of these cations.

A further rise in the amount of LPO products in the clusters (fig. 3) can lead to fragmentation and destruction of sarcolemmal and sarcoplasmic reticulum membranes. Hence, this effect first modifies the functions of the cardiomyocyte membranes and subsequently becomes the cause of membrane destruction.

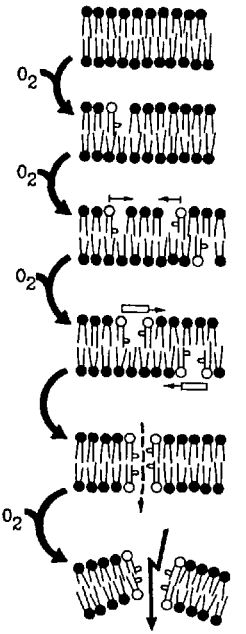


Fig. 3. Formation of lipid peroxide clusters and membrane fragmentation upon induction of lipid peroxidation. For details see Text.

When attempting to elucidate the mechanism of LPO activation as manifested by its damaging action on cell membranes, and by myocyte death, in stress, ischemia and reoxygenation, one should bear in mind that this lipid triad component is closely connected with the other components and pathogenetic links of ischemic heart damage. For instance, LPO activation causes labilization of the lysosomal system, in which the bulk of cell phospholipases is localized. The phospholipases which are released from the lysosomes as well as the activated membrane-bound phospholipases can play an important role in destruction of the membrane lipid bilayer and in formation of lysophospholipids and free fatty acids (30). The lysophospholipids and high concentrations of fatty acids occurring in the blood in stress and infarction can destroy the highly ordered phospholipid arrangement of the phospholipid membrane through their detergentlike action and thus induce LPO activation. The increase of membrane permeability for Ca^{2+} resulting from this chain of reactions, and the thus produced Ca^{2+} excess in the cell, activate in turn the phospholipases and LPO (23, 57). This results in closure of the vicious circle which plays a crucial role in the development of cell membrane damage, the conversion to irreversible damage, and myocyte death.

In practically every cell of an aerobic organism there are conditions conducive to LPO induction. However, damaging activation of LPO, or cell membrane destruction, does not occur in healthy organisms. In routine stress situations and in relative hypoxia caused by considerable

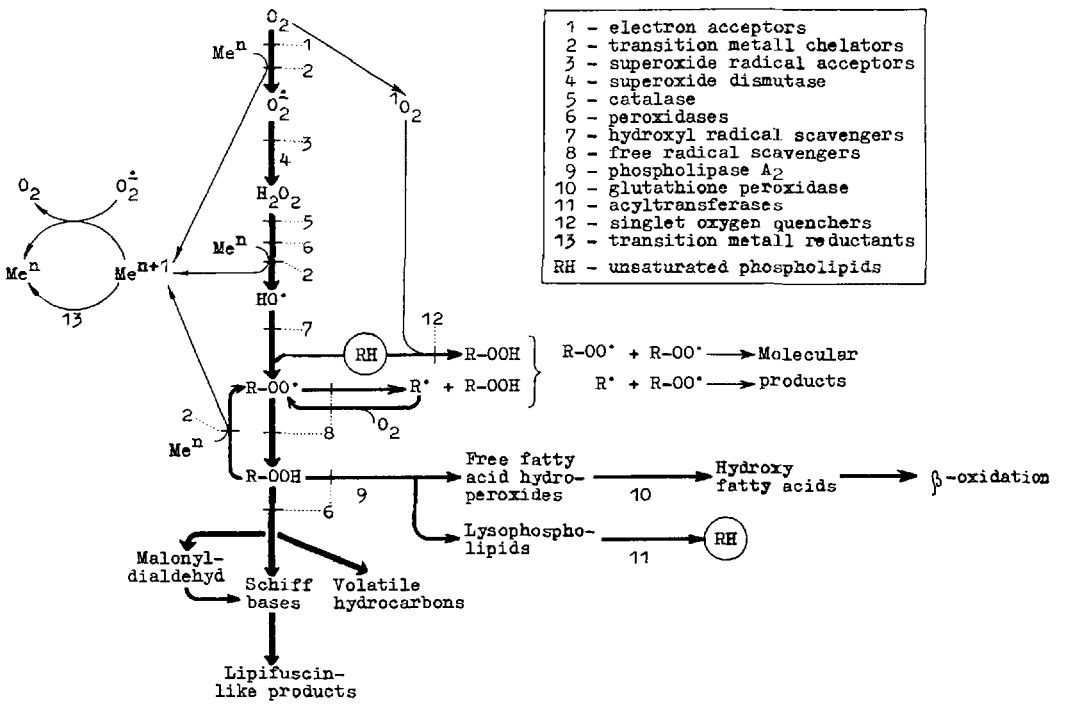


Fig. 4. A schematic representation of the antioxidative system of the cell.

physical load this activation is quite limited and does not cause irreversible damages.

Such limitation, which provides for active behavioral responses of an organism in complex environmental situations, is due to the existence of a continuously functioning, reliable antioxidative system which restricts practically all the steps of the LPO process (fig. 4).

The antioxidative system only proves to be insufficient under excessive (with respect to duration and intensity) stressory influences, chronic anoxia, ischemia and subsequent reoxygenation. The result is enhanced LPO activation, development of the whole lipid triad and irreversible damage. It is exactly in such situations that effective protection of the heart and other organs can be achieved by administration of LPO inhibitors, i.e., natural and synthetic free radical scavengers (antioxidants). The group of natural antioxidants may be associated with vitamins of the E and K groups, steroid hormones, biogenic amines, sulfur-containing amino acids, etc. The most effective synthetic free radical scavengers are LPO inhibitors of the phenolic type, e.g., butylated hydroxy toluene (BHT), butylated hydroxy anisole, hydroxypyridine derivatives, etc. Further on we shall consider the use of one of such compound, BHT, for prevention of hypoxic, ischemic and reoxygenation damages of the heart.

The use of antioxidants for preventing damage caused by experimental infarction and reoxygenation

Acute myocardial ischemia caused by occlusion or spasm of the coronary artery is characterized by a number of factors which, when acting simultaneously, may induce LPO activation.

Indeed, accumulation of reduced carriers by switch-off of the respiratory chain is concomitant with the presence of molecular oxygen. The O_2 tension does not fall below 5–10 mm Hg even in the focus of ischemia; and in natural reoxygenation resulting from cessation of the spasm or from reactive hyperemia O_2 tension may be considerably elevated. Hence the two main conditions necessary for free radical formation and LPO induction, i.e., excess of electron donors – reduced carriers – and the electron acceptor – O_2 – are given in the case of ischemia. Furthermore, accompanying emotional, painful stress provides for increased release of catecholamines. Under these conditions epinephrine oxidation to adreeochrome can give rise to epinephrine semiquinone, which can donate electrons to O_2 and thus generate a superoxide anion radical (8), an inducer of LPO.

In accordance with the above considerations it has been demonstrated that emotional, painful stress, which is originally not associated with ischemia, can cause a manyfold increase of LPO products in the myocardium (45). It should thus be expected that ischemia in combination with reoxygenation and stress, which is the case in any acute myocardial infarction, should lead to strong activation of LPO in heart muscle.

Indeed, 24 hours following the onset of myocardial infarction caused by ligation of the descending left coronary artery of the rat, the thermochemiluminescence of lipids in the ischemic zone of the myocardium is increased 3–4-fold – an indirect indication of LPO activation. The increase

reaches its maximum on the 3rd day after coronary artery ligation and gradually disappears by the 30th day. The degree of thermochemiluminescence activation, the size of the infarction zone, and typical ischemic changes in the ECG can be considerably reduced by preliminary administration of antioxidants – sodium selenite, α-tocopherol and the combination of these two drugs (31). Evidence for this notion was also obtained in the laboratory of the present authors (26, 27).

When evaluating this important data, one should consider that the thermochemiluminscence of lipids isolated from the heart reflects the LPO process occurring in vitro under aerobic conditions. Consequently, the increased thermochemiluminescence of lipids obtained from the ischemic zone is only suggestive of the fact that ischemia creates prerequisites for the more intensive occurrence of this process in the presence of O_2. This shift is not an unequivocal proof of LPO activation in vivo; such activation may also be reflected in increased content of unsaturated fatty acids, substrates of LPO, and lipids of the ischemic zone; or by decreased activity of natural antioxidative systems, etc. In order to find out whether or not LPO activation takes place in the ischemic or extraischemic zone, it is apparently necessary to measure the content of LPO products accumulated in myocardial lipids as a result of LPO activation. We thus determined the content of primary (hydroperoxides) and end products of LPO (Schiff bases) in the ischemic and extraischemic zones 24 hours after ligation of the descending left coronary artery of the rat, i.e., under conditions of experimental myocardial infarction. The state of enzymatic antioxidative systems in these zones was evaluated simultaneously (43).

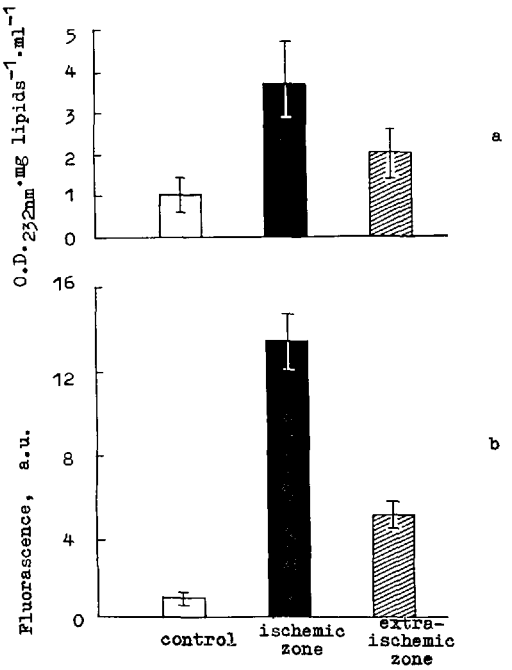


Fig. 5. Accumulation of lipid hydroperoxides (a) and Schiff bases (b) in heart muscle in experimental myocardial infarction.

The diagram shown in figure 5a demonstrates that 24 hours after coronary artery occlusion the hydroperoxide content in the ischemic zone is increased 3.5-fold, that in the extraischemic zone 2-fold.

As can be seen from figure 5b, the content of Schiff bases, the end products of LPO, in the ischemic and extraischemic zones is increased 13-fold and 5-fold, respectively. Such excessive activation of LPO in myocardial infarction is accompanied by decreased activity of the main enzymatic antioxidative systems which restrict the intensity of LPO under normal conditions.

Figure 6 (a–c) shows that the activity of the two antioxidative enzymes, superoxide dismutase and catalase, restricting the production of activated oxygen species is reduced by 16–18 %, while that of glutathione peroxi-

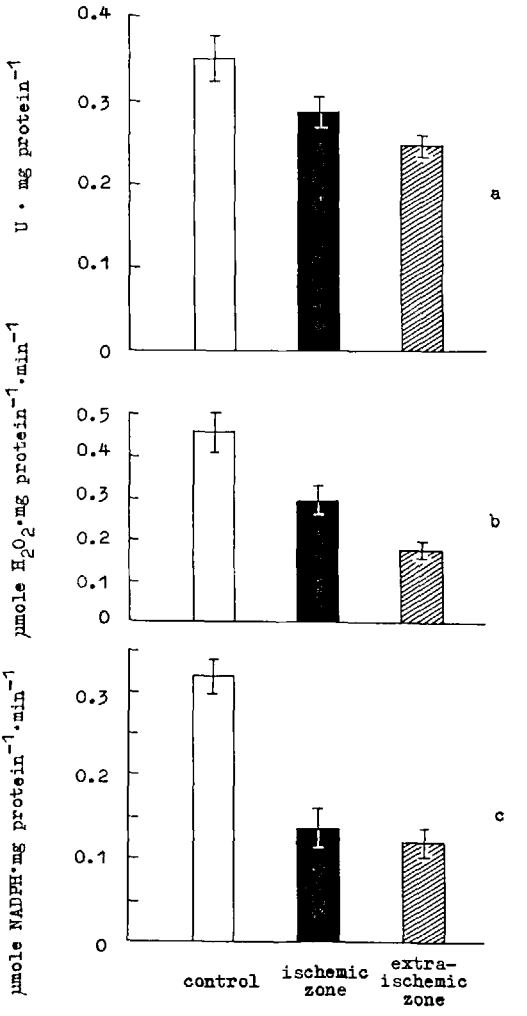


Fig. 6. Decrease in the activity of the main antioxidative enzymes of heart muscle in experimental infarction: a – superoxide dismutase, b – catalase, c – glutathione peroxidase.

dase, which responsible for utilization of lipid hydroperoxides, is decreased more than 2-fold.

Thus, experimental myocardial infarction is characterized by a considerable accumulation of primary and end products of LPO and by partial inactivation of certain enzymatic systems which restrict the LPO process under normal conditions. It should be noted that such an extensive activation of LPO in myocyte membranes containing polyenoic phospholipids, the main substrates of LPO, is in no way fortuitous; this process is inevitably associated with activation of phospholipases and detergent-like action of fatty acids and lysophospholipids. This lipid triad should, in its turn, cause extensive destruction of the membrane lipid bilayer.

These considerations are in good agreement with the latest reports (12). It has been shown that 12 hours after the onset of ischemia the total phospholipid content in the heart is decreased by $^1/_3$, while there is a 50 % fall in the content of the main substrate of LPO, phosphatidyl ethanolamine, and only a 25 % fall of phosphatidyl choline. Such a loss of membrane phospholipids is associated with a 10-fold increase of sarcolemma permeability for Ca^{2+}. This is only natural, since the most significant decrease of the phospholipid content takes place in the sarcolemma, where the destruction of the lipid bilayer probably occurs at the earliest stages (56).

There is same evidence that activation of LPO and destruction of the lipid bilayer of heart muscle membranes can take place not only in experimental infarction, but may occur in human subjects as well. Clinical investigations by E. R. Katsanovich (29) demonstrated that the content of lipid hydroperoxides in the blood under acute myocardial infarction is increased more than 4-fold. This is in agreement with data from earlier studies (19) suggesting that the cardiomyocytes of autopsied myocardial infarction patients have a decreased content of phosphatidyl ethanolamine, the main substrate of LPO.

The experimental data given above demonstrate that the LPO process in myocardial infarction is not activated in the ischemic zone alone; it is considerably increased in the extraischemic zone as well. The activation in the extraischemic zone is probably due to stress, on the one hand, which causes catecholamine excess, and to natural reoxygenation resulting from reactive hyperemia and collateral opening, on the other hand. It is also possible that such activation associated with decreased activity of the antioxidative systems can be responsible for the enlargement of the myocardial necrotic zone underlying the so-called recurrent or prolonged infarctions which have been widely reported. In light of this notion it seems worthwhile to examine the recent results on the use of free radical scavengers in cardiological practice aimed at myocardial protection against experimental infarction, transitory ischemia, hypoxia and hypoxic contracture.

The data presented in table 1 show that a preliminary injection of the antioxidant did not significantly affect the size of the infarction zone 48 hours after ligation of the left coronary artery descending branch. BHT only decreased the degree of fermentemia associated with the infarction; the same effect of BHT was, however, observed in the case of emotional-painful stress. It may therefore be assumed that the fermentemia observed

Table 1. Effect of preliminary injection of BHT on the size of ischemic necrotic zone and degree of fermentemia after ligation of the left descending coronary artery of the heart (M ± m).

	Infarction (n = 11)	BHT + infarction (n = 9)	P
Square of necrotic zone under the epicardium/square of the left ventricular wall, %	63.3 ± 2.0	59.5 ± 3.1	> 0.05
Square of necrotic zone under the endocardium/square of the left ventricular wall, %	50.9 ± 1.9	43.7 ± 3.2	> 0.05
Activity of aspartate amino-transferase in blood serum, U/l	130.4 ± 12.2	97.2 ± 7.1	< 0.05

48 hours after the onset of myocardial infarction is mainly due to stress damage of various tissues and its decrease is a result of the antistressory effect of BHT.

The curves shown in figure 7 reflect the dynamic of the pressure developed in the left ventricle during relative rest and at maximal load caused by tension after 25-sec clamping of the aorta. In animals with

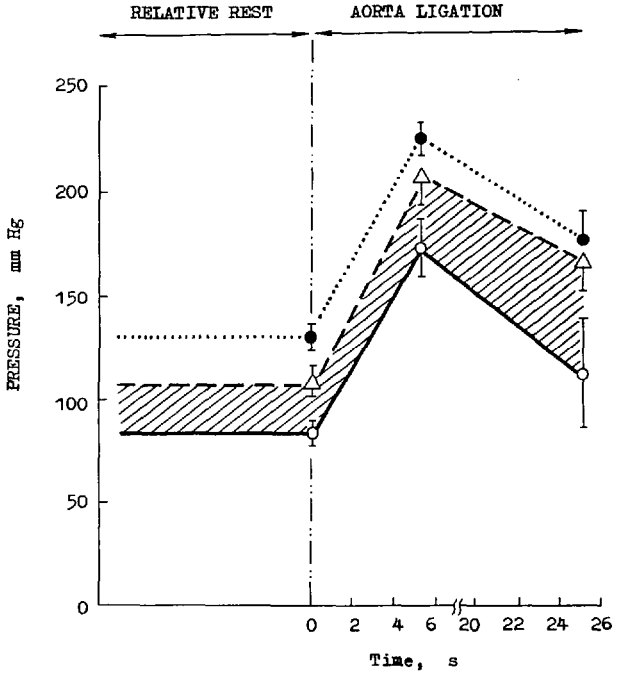


Fig. 7. Effect of preliminary injection of the free radical scavenger BHT on the impairment of left ventricular contractility in experimental infarction:
○ – control; ● – infarction; △ – BHT + infarction.

experimental infarction the value of this pressure and the velocity of its development are significantly decreased as compared to the control. This depression caused by infarction is maximal at the 25th second after aorta ligation when an fatigue of the safe part of the myocardium is developed. The most essential experimental finding consists in the fact that a preliminary injection of the antioxidant considerably prevents the development of this contractile function damage. The hatched area in the figure 7 designates the protective response.

Since the size of the ischemic necrotic zone is decreased by the antioxidant only insignificantly, we have supposed that this effect may be due to the antioxidant protection of the safe, non-ischemic zones of the myocardium against the stress-induced damages concomitant with myocardial infarction. In other words, it seems probable that in this case we deal with a protection of the intact divisions of the myocardium against stress damage. To verify this assumption we studied the contractility of the experimentally non-ischemic zones of the heart, namely the right auricle of animals with experimental infarction of the left ventricle.

The table 2 shows that 24 hours after the onset of experimental infarction in rat left ventricle the contractile function of the right auricle is strongly disturbed, showing in a 2-fold decrease of the maximal pressure developed by the isolated auricle during isometric contraction. A preliminary injection of the β-blocker inderal prevents this defect of the contractile function. Consequently, the auricular damage is due to catecholamine excess, that is, to adrenergic stress effect associated with infarction. The table 2 also demonstrates that BHT prevents the disturbed contractile function of the auricle in the same degree as inderal. In this way the antioxidant prevents the depression of contractile function of the non-ischemic part of the heart under acute period of myocardial infarction and the antioxidant protection of the heart is in this case an antistressory one.

Traditionally, protection of the heart against experimental infarction is a model of prevention of myocardial infarction in man and, correspondingly, heart protection against experimental transitory ischemia is a model of prevention of coronary attacks.

Figure 8 shows the dynamics of pressure developed in the left ventricle and the intensity of functioning of structures after 30 min occlusion of the left descending coronary artery of the rat, and the effect of BHT on this process. It is evident that ischemia causes considerable depression of heart contractility and subsequent stabilization at a low level; this data is

Table 2. Effect of preliminary inderal or ionol administration on contractile function of heart auricle in experimental left ventricle infarction.

Series	Developing tension, mg (n = 9)
Control	382 ± 13
Infarction	198 ± 5*)
Inderal + infarction	331 ± 15
Ionol + infarction	315 ± 15

*) $P < 0.01$

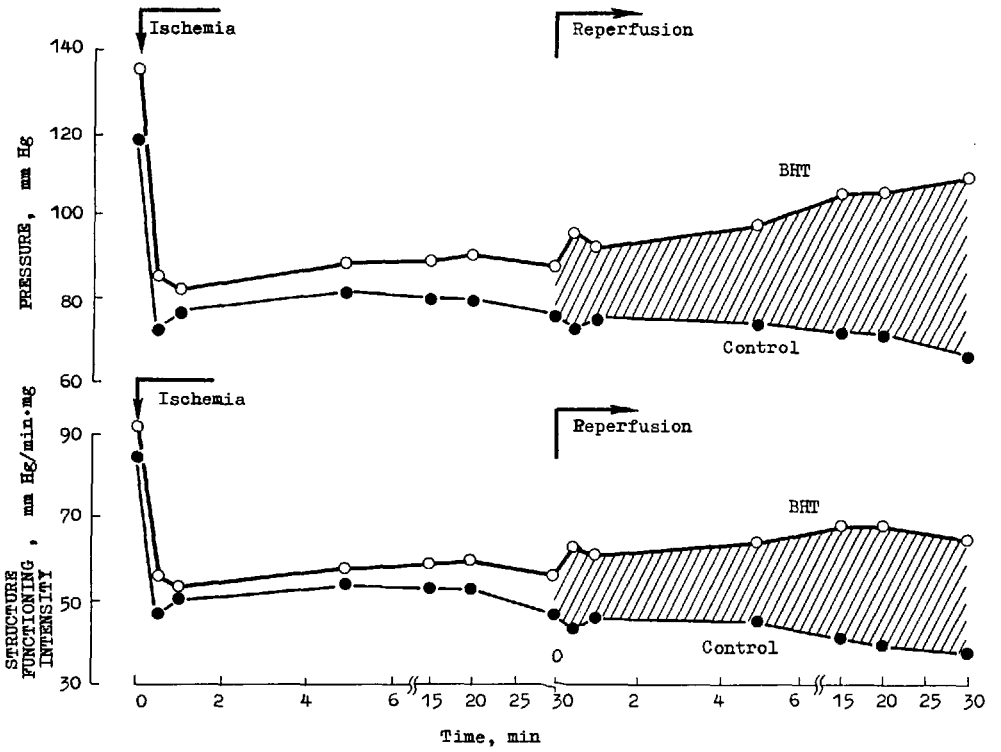


Fig. 8. Effect of the free radical scavenger BHT on the depression of left ventricular contractility in transitory ischemia and subsequent reperfusion.

supported by reports from other laboratories (39). Removal of the coronary artery occlusion and further reperfusion and reoxygenation of the myocardium fail to restore the contractile function, and even cause further slow depression which, in all probability, is due to the maximal activation of LPO and membrane destruction during reoxygenation.

It is of interest that the animals receiving a relatively low (20 mg/kg) dose of BHT prior to experimental transitory ischemia showed practically the same response to acute ischemia as the controls; however, BHT completely prevented the reoxygenation depression of contractile function and, moreover, provided for considerable restoration of heart contractility during reoxygenation.

This data reveal a prerequisite for the possible use of synthetic free radical scavengers in the therapy of coronary disease and indicate that the inhibitors of free radical oxidation provide for exceedingly effective protection of the heart under reoxygenation conditions, when anoxia is followed by a sufficient or excessive supply of O_2. The latter observation was convincingly confirmed by the use of free radical scavengers for heart protection against hypoxia, posthypoxic reoxygenation and, finally, hypoxic contracture.

Table 3. Dynamics of Schiff base content in myocardium under hypoxia and subsequent reoxygenation (fluorescence intensity in arbitrary units).

Exptl. animals	Artificial respiration (aerobic conditions)	Cessation of respiration (hypoxia)	Resumption of respiration (reoxygenation)
Control	8.1 ± 2.0	13.4 ± 2.2	16.0 ± 3.2
BHT	3.9 ± 1.9	3.3 ± 2.4	3.04 ± 0.99

Recent studies have demonstrated that preliminary administration of free radical scavengers has no substantial influence on the impaired contractility of animal heart caused by cessation of artificial respiration, but significantly affects contractile function in the course of subsequent reoxygenation. While the restoration of this function is slow and incomplete in control animals, contractility is restored immediately after resumption of respiration, and is inevitably associated with superrestoration, in animals pretreated with antioxidants (42).

Further analysis demonstrated that the ability of antioxidants to prevent reoxygenation damage to heart contractility and to provide for superrestoration of this function after cessation of hypoxia is based on inhibition of LPO, as may be expected.

The data of table 3 suggest that 5 min hypoxia alone causes an insignificant increase in the content of LPO end products in myocardium; upon reoxygenation the content is considerably increased and excedes the original level by 2-fold. BHT decreases the fluorescent Schiff base content even under conditions of normal respiration, and completely prevents accumulation of such constituents in hypoxia and reoxygenation.

The postischemic and posthypoxic reoxygenation damage which is effectively prevented by antioxidants, as well as hypoxia itself, plays an important role in the pathogenesis of heart disease. In open heart surgery for instance, the restoration of the blood supply impaired by prolonged hypoxia of the myocardium can cause reoxygenation contracture of the human heart; the resulting "stone heart" (14) effect underlies acute cardiac failure which develops in the course of surgery. It therefore seems essential to evaluate the possible use of antioxidants for heart protection against hypoxic contracture under conditions in which the amount of available O_2 is absolutely insufficient for the respiratory resynthesis of ATP required for normal contractile function of the heart, but is sufficient to produce LPO activation when the reduced carriers are accumulated in a partially inhibited respiratory chain of mitochondria. Such a contracture can virtually be reproduced on the isoalated isovolumic heart. Our data demonstrated that a preliminary injection of the watersoluble antioxidant HP-6 to rats provided almost complete protection against hypoxic contracture.

In this way, the inhibition of LPO prevents both posthypoxic damage of heart contractility in reoxygenation and the development of hypoxic contracture. It should be noted that the contracture which results from a more severe hypoxia under similar conditions, i.e., almost complete anoxia of the isolated heart, cannot be prevented by free radical scavengers (40).

In other experiments in the isolated heart, both BHT and HP-6 were injected into the animals for 3 days prior to heart excision and failed to prevent the hypoxia-induced depression of contractility. But the agents did sharply increase the rate and degree of resumption of contractile function during reoxygenation (42).

Our results are in agreement with other biochemical data (17, 18) suggesting that molonic dialdehyde, a LPO product, does not accumulate in the myocardium in chronic anoxia of the isolated heart; upon subsequent reoxygenation, malonic dialdehyde is rapidly accumulated in amounts proportional to the duration of preceding anoxia. For instance, the malonic dialdehyde content is increased 4-fold under conditions of 80 min anoxia with subsequent reoxygenation.

Thus, the data suggest that the antioxidants – free radical scavengers – protect the heart muscle only in case of excessive LPO activation, namely, under stress, reoxygenation, and, possibly, in moderate hypoxia. In severe ischemia and anoxia, when the amount of O_2 dissolved in membrane lipids is insufficient to produce LPO activation, this activation and, correspondingly, the protective effects of antioxidants are either not proven or altogether absent. This, however, does not contradict the fact that preliminary injection of antioxidants limits the size of experimental myocardial infarction. It has already been mentioned that this protective effect can be due to a switch-off of the stressory and reoxygenation components of heart damage or to a possible increase of resistance of the peripheral infarction zone to mild hypoxia. Successful application of free radical scavengers to the therapy of heart ischemia not only depends on the proper choice and reasonable dosage of free radical scavengers, but also on the well-balanced combination of such agents with other factors as well. The above-described pathogenetic chain of ischemic heart damage suggests that the most promising are the following combinations are those of the antioxidants with a) central inhibitors of the stressory response (tranquilizers, GOBA, seduxen, tazepam, elenium); b) β-blockers; c) lipase and phospholipase inhibitors; and d) factors blocking the slow electrogenic channel of Ca^{2+} transport (verapamil, dilthiazem nifidepine).

Conclusions

It may be concluded that in the whole organism subjected to myocardial infarction the activation of LPO and other components of the lipid triad which are involved in irreversible destruction of the cardiomyocyte membranes, is not due to the effect of ischemia alone, but to the combined action of stress, reoxygenation and ischemia. Free radical scavengers suppress LPO activation during stress, reoxygenation and probably in moderate ischemic hypoxia and can therefore limit the size of the ischemic necrotic zone of myocardium. The use of natural and synthetic free radical scavengers in the therapy of ischemic disease and their combination with other factors protecting the heart against ischemic damage is of great practical importance and should be examined in clinico-physiological trials.

Zusammenfassung

In der vorliegenden Studie wird eine Arbeitshypothese zur Pathogenese der ischämischen Herzschädigung aufgestellt. Nach dieser Hypothese ist ein entscheidender Faktor für den Übergang von der reversiblen zur irreversiblen Schädigung eine Membrandestruktion der Herzmuskelzellen, welche durch sog. Lipid-Triaden verursacht wird. Letztere beinhaltet eine Aktivierung der Lipid-Peroxidation und der Phospholipase sowie eine Detergenz-ähnliche Wirkung exzessiver Mengen von freien Fettsäuren und Lysophospholipiden. Eine ausgeprägte Aktivierung der Lipid-Peroxidation beim experimentellen Herzinfarkt sowie eine Reoxigenierung nach vorübergehender Ischämie wurden demonstriert. Die vorgelegte Hypothese und die experimentellen Daten legen eine Anwendung von Radialfängern (Antioxidantien) nahe für die Protektion des Herzens gegen experimentellen Myokardinfarkt, vorübergehende Ischämie und emotionalen Streß bei Schmerzsituationen.

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