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LIPID PEROXIDATION IN CHILDREN WITH DUCHENNE'S HEREDITARY MUSCULAR DYSTROPHY

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One of the most urgent problems in molecular neurobiology is the formation of a clear idea of how membranes really perform their physiological functions, what affects them, and how such knowledge can be utilized in the treatment of severe human neuromuscular diseases.

As a model with which to study the effect of peroxidation (LPO) on membrane permeability in such patients, it was decided to study a hereditary disease, namely Duchenne's progressive hypertrophic muscular dystrophy (PHMD), which has a particularly severe course and semilethal outcome.

In 1958, on the basis of research showing the unusually high activity of certain sarcoplasmic enzymes in the blood serum of patients with PHMD [6], the hypothesis of a lesion of the muscle fiber membranes was first put forward [6]. Subsequent biochemical and morphological studies confirms this hypothesis not only for the sarcolemma, but also for membranes of the sarcoplasmic reticulum (SR) and mitochondria [7, 12, 13]. However, the concrete mechanisms of damage to the muscle cell membranes has not yet been explained.

Considering the universal role of LPO products in membrane lesions of various cells, including muscle cells, it was decided to examine the possible role of LPO in the pathogenesis of PHMD. In the present investigation the content of gaseous LPO products (pentane) was studied in samples of expired air in patients with PHMD.

EXPERIMENTAL METHOD

The subjects studied comprised 18 boys with PHMD, in whom the diagnosis had been made after further clinical, electrophysiological, genealogic, and biochemical analysis. The control group consisted of clincially healthy boys of the same age with no family history of neuromuscular diseases. According to the degree of development of the muscular dystrophy the patients were divided into three groups: 1) minimal clinical manifestations of muscular dystrophy but with maximal activity of sarcoplasmic enzymes in the serum (especially creatine phosphokinase — CPK), 2) with a picture of clinically manifest muscular dystrophy and with a moderately increased CPK activity, 3) maximal clincial manifestations with inability to walk unaided and with low serum CPK activity. The children's mental state was assessed by psychological tests, school reports, and conversations with the child and parents. CPK activity

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TABLE 1. Results of Clinical and Biochemical Investigations on Boys with Duchenne's Muscular Dystrophy

Surname, forename	Age	Stage	Mental state	CPK activity, IU	Pentane concentration
1. R-ko, Vitya	6	1	Retarded	25	253
2. Kh-n, Vitya	5	1	Backward	45	224
3. B-n, Alesha	6	2	Feeble minded	45	103
4. N-v, Lenya	11	2	No data	15	71
5. V-v, Kir	10	2	»	28	220
6. M-ch, Andrei	12	2	»	30	180
7. Kh-v, Bakhtiyar	8	2	Backward	38	289
8. T-v, Alesha	10	2	No data	18	97
9. G-i, Edik	11	2	No data	15	81
10. P-k, Sasha	8	2	Retarded	45	231
11. F-v, Vanya	4	1	»	68	122
12. G-v, Zhenya	8	2	»	52	122
13. D-v, Borya	8	2	»	70	289
14. B-v, Andrei	10	3	Backward	18	118
15. E-v, Roma	10	3	»	10	81
16. Sh-v, Kostya	11	3	»	9	98
17. S-v, Slava	11	3	»	10	159
18. K-v, Alesha	13	3	»	5	62
Mean	9 years	1—3	Normal to backward	30 ±4,9	160,5 ±18,8
Mean for normal boys	9 years		Normal	0—1,5	43±4,2

was determined by the method suggested previously [2]. A method of determination of gaseous LPO products was used. Quantitative analysis of hydrocarbons in the expired air was based on the assumption that the principal substrate for LPO consists of ω-6-polyunsaturated fatty acid residues of phospholipids and, correspondingly, the principal gaseous LPO product, present in the largest quantities, is pentane. Accordingly, to ensure maximal sensitivity of the method, the pentane concentration was determined in the expired air [9]. Expired air from the affected children was collected in 2-liter polyethylene bags, flushed out beforehand with pure nitrogen. Organic substances contained in the expired air were concentrated on a 4 × 40 ml column packed with TENAS-GC 60-80 mesh (The Netherlands), a sorbent which traps organic substances and allows inorganic to pass through. For this purpose air from the bag was passed through the column for 10 min at the rate of 30-40 ml/min. The absorbing column with gas chromatography system was then connected. The carrier gas, having passed through the absorption column, entered the Y-shaped capillary tube of the chromatograph injector. After the carrier gas had been blown through for 5 min, the capillary tube was immersed in liquid nitrogen and the absorption column heated electrically for 15 sec to 300°C. After 3 min the capillary tube was heated for 50-60 sec to 250-300°C. Chromatographic analysis was carried out on a Perkin-Elmer F-22 Gas Chromatograph (Sweden) with flame-ionization detector. Glass columns measuring 3 mm × 2 m were packed with Activated Alumina F1, 40-60 mesh (from the same firm). Highly pure helium (USSR) was used as the carrier gas. The flow rates were: helium 15 ml/min, hydrogen 40 ml/min, air 40 ml/min. The temperature of the column was 160°C, of the injector 210°C, and of the detector 220°C. The area of the peaks was integrated on a M-1 Computing Integrator (from the same firm). The pentane outflow time was 5-6 min. The error of the method did not exceed 10%. The pentane concentration in the expired air was expressed in picomoles per liter.

The experimental results are summarized in Table 1, which gives the principal information about each child taking part in the study, and in which two stages of the disease, II and III, are compared.

EXPERIMENTAL RESULTS

The results (Table 1) showed that in patients with PHMD the pentane concentration in the expired air was 4 times higher than the mean values for children of the control group.

Incidentally, correlation was not present between the plasma CPK activity and pentane concentration in the expired air of these children (r = 0.56).

Examination of children with stage I-II of the disease and with stage III separately demonstrates that the pentane concentration was higher in the early stages of the disease: 182 ± 22.7 pmole/liter for stages I-II and 103.6 ± 18.6 pmoles/liter for stage III. It is at these stages of PHMD that the dystrophic process becomes fully manifested, with massive death of muscle cells and their replacement by adipose and connective tissues.

The most characteristic biochemical feature of PHMD is a change in lipid metabolism: a decrease in the concentration of unsaturated phospholipids (phosphatidylethanolamine, phosphatidylcholine) and an increase in the content of saturated phospholipids in the various membranous structures of the muscle cells [3, 8, 10, 11]. Correspondingly, in the fatty acid composition polyunsaturated ($C_{20:4}$, $C_{22:6}$) fatty acids are replaced by saturated and monoenic fatty acids. Considering that it is these polyenic aliphatic acyl groups that are the principal substrate for lipid peroxidation, it must be concluded that these changes in the phospholipid composition and fatty acid spectrum may be linked with activation of LPO in PHMD.

It was shown previously in studies both *in vivo* and *in vitro* that LPO is an effective modifier of the sarcoplasmic reticulum of skeletal muscles and induces inhibition of Ca-transporting capacity, which causes a disturbance of contractile activity of the muscles [1, 4, 5]. In this connection it may be recalled that a characteristic feature of the late stages of PHMD is the development of muscular contractures.

The effect of marked activation of endogenous LPO observed in Duchenne's muscular dystrophy points to its possible participation in the pathogenesis of this disease.

However, the pathogenetic role of free-radical LPO reactions can be definitively determined only by quantitative comparison of the ability of LPO inhibitors to prevent accumulation of lipid peroxides and at the same time, to abolish the characteristic biochemical and clinical manifestations of the disease. Future research will be devoted to a study of these problems.

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