# LIPID PEROXIDATION AND MYELOPEROXIDASE ACTIVITY OF NEUTROPHILS IN TURNER'S SYNDROME

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Turner's syndrome (TS) is characterized by hormonal imbalance and is accompanied by several biochemical disturbances affecting different systems and tissues. A characteristic feature of TS is premature aging, which differs from physiological aging in its rapid progression. The writers previously discovered a disturbance of lipid metabolism and a sharp increase in the frequency of hyperlipidemias in these patients [1].

The object of this investigation was to continue the study of one aspect of lipid metabolism in patients with TS, notably the state of the lipid peroxidation (LPO) system in blood neutrophils. In the healthy individual, the normal level of lipid peroxides is maintained through the participation of antioxidant systems. A component of one of these systems is myeloperoxidase (MPO), an enzyme concerned with the catabolism of organic and inorganic peroxides present in sufficiently high concentrations in neutrophils and blood serum. It is possible that in TS the depressed functioning of certain antioxidant systems and, in particular, of the MPO system may lead to the accumulation of lipid peroxides in the cells.

## EXPERIMENTAL METHOD

Venous blood with heparin was used. A leukocyte suspension was isolated by the addition of 3% gelatin solution made up in physiological saline. To remove erythrocytes, the cell residue was treated with $NH_4Cl$. The washed cells were suspended in 1 ml of 0.1 M phosphate buffer (pH 7.0), disintegrated in a glass homogenizer, and used to determine the level of LPO products. To study MPO activity, the cells were disintegrated in a type UZDN-1 ultrasonic disintegrator at 22 kHz for 30 sec. The intensity of LPO was judged by the content of products reacting with 2-thiobarbituric acid (TBA) [7]. Leukocyte homogenates were incubated for 1 h at 37°C: with the addition of 0.25 mM NADPH and without its addition (to determine the intensity of spontaneous LPO). Total lipids were determined by a modified method in [3]. To investigate MPO activity, the method in [9] was used and protein was determined quantitatively by Lowry's method [6]. To investigate the temperature sensitivity of MPO samples were incubated for 10 min at 80°C, after which enzyme activity was measured. Preliminary experiments showed that these are the most demonstrative conditions for MPO assay.

## EXPERIMENTAL RESULTS

Data on the intensity of LPO and also the activity and thermostability of MPO in the neutrophils of patients with TS are given in Table 1. Clearly, in patients with this syndrome the level of TBA-active products was higher than the control both for spontaneous and for NADPH-dependent peroxidation, and MPO activity was low. These results indicate that LPO is intensified in TS, in agreement with the observed disturbance of the functional properties of MPO.

Changes in enzyme activity are known to arise for different reasons. In particular, they may be due to disturbances of protein structure. To study this possibility, an increase in the sensitivity of proteins with modified physicochemical properties to temperature was used [2, 8]. Analysis of temperature sensitivity of MPO revealed a decrease in the thermostability of the enzyme in patients with TS. A similar observation of lowered thermostability of a number of blood cell enzymes has been demonstrated in several diseases accompanied by more rapid aging

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TABLE 1. Content of LPO Products, Activity and Thermostability of MPO in Blood Neutrophils of Patients with TS (M ± m)

| Group of subjects | Concentration of TBA-active products, μmoles mg lipid | | Specific MPO activity, conventional units/mg protein | Thermostability of MPO, % |
|---|---|---|---|---|
| | NADPH-dependent LPO | spontaneous LPO | | |
| Patients with TS | 2,53±0,39* (14) | 1,66±0,08*** (14) | 2286,0±242,9* (29) | 54,5±3,4** (29) |
| Control | 1,4±0,18 | 0,96±0,14 | 3096,0±317,2 | 68,3±2,9 |

Legend. *P < 0.05, **P < 0.01, ***P < 0.001 (in all cases compared with control); number of patients tested given in parentheses.

(progeria, Down's syndrome). In the opinion of the authors cited, this is due to post-translation modification of protein molecules [8].

Changes in the level of TBA-active products and MPO activity develop against the background of a hormonal imbalance that is characteristic of TS. Activity of peroxidases, including MPO, has been shown to be stimulated by hormones and, in particular, by estrogens [9]. Estrogen production is known to be sharply reduced or completely absent in patients with TS. Of 29 patients with this syndrome under the writers' observation, 10 were taking estrogens when they were investigated; in eight of them the level of MPO activity was within normal limits. Estrogen therapy did not have a normalizing action on the thermostability of this enzyme. There is also evidence that estrogens can stabilize MPO and prevent its denaturation in the presence of high concentrations of peroxide derivatives [4].

The increased level of LPO production and changes in the activity and thermostability of MPO are thus evidence of the functional defectiveness of the neutrophils in patients with TS. The changes discovered reflect a general process of aging, which spreads to the blood cells. The disturbances observed are probably based on an abnormal set of sex chromosomes and a connected disorder of hormonal regulation.

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