INFLUENCE OF THYMUS EXTRACT (TFX) ON LIPID PEROXIDATION IN THE PLASMA OF RATS FOLLOWING THERMAL INJURY

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Lipid peroxidation products in burn alone and after TFX treatment were assessed. Obtained results showed that lipid peroxidation process began immediately after severe burn and was continued during the whole 48 h post burn period. Burn enhanced the lipid peroxidation products and the TFX treatment slightly diminished the malonyldialdehyde (MDA) and conjugate dienes (CD) plasma concentration. Our investigations suggest that TFX treatment enhanced the post burn lipid hydroperoxides (HPETE) generation. This effect may be the consequence of modulation of immune system, which causes the enhanced activity of lipoxygenase and simultaneously decrease in cyclooxygenase activity after TFX treatment. TFX treatment one hour after burn prevented the ongoing post burn peroxidation 24 and 48 h after burn. The observations suggest that it may be helpful to improve the peroxidation - antioxidation imbalance which is the consequence of severe burn.

Key words: rats, burn wound, thymus extract, TFX, lipid peroxidation.

Reactive oxygen forms (ROF) or free radicals are the atoms or molecules containing unpaired electrons on the last shell what makes them highly reactive and responsible for oxidation damage of lipids (1, 26). Peroxidation of lipid forming cell membranes causes changes in their structure and, in turn, the dysfunction of affected cells. In consequence, they cause burn oedema, intravascular haemolysis, hypovolemic shock, an Adult Respiratory Distress Syndrome or Multiple Organ Failure Syndrome (2, 5, 15). Lipid peroxidation process occurs after severe burn and is continued during the whole 48 h post burn period. These processes can damage the lipid part of the biological membrane which leads to the loss of its function (6) and apoptosis.

Lipid peroxidation being a most spectacular manifestation of cell damage causes the transformation of membrane polyunsaturated fatty acids to hydroperoxides and degradation to low molecular species, respectively. One of the most important of these products is malonyldialdehyde (MDA) which is an indicator of free radical activity.

Oxygen radicals belong to the causative agents responsible for the development of burn shock and distant organ injury in animal models of burn trauma.
Patients with burns demonstrated evidence of increased oxygen free radical activity and activation of polymorphonuclear cell and macrophages (22). In all cases of this trauma, neutrophils diffusely invade these organs being the potent source of reactive oxygen metabolites. It leads to enhancement of lipid peroxidation products (MDA, CD) in plasma which may be an indicator of post burn free radicals generation.

The thymus hormones were reported to be effective on lipid peroxidation and the antioxidant system (11). The ability of thymus hormone of 5-thymosine fraction and TFX to enhance the lymphokine IL-2 and interferon gamma production, and host antioxidant enzymes activity - both diminished the lipoxygenation (28). Gokkusu et al. mentioned that thymosine a1 might normalize changed lipid levels and increased lipid peroxides (12).

The aim of this study was to assay the effect of TFX treatment in burn on the lipid peroxidation intensity in the rat plasma.

Material and Methods

Male Wistar rats with body weight 250g ± 50g, obtained from the Laboratory of Department of Pathophysiology Medical University in Lublin were used in the study. The experiment was carried out in accordance with guidelines of Animal Ethical Research Committee of Medical University in Lublin.

The animals were divided into three groups. The control group were rats anaesthetized with Thiopental (50 mg/kg of body weight) and left without further treatment. The 2nd group were rats with third-degree burn of 30% of the body surface. The animals were anesthetized with intraperitoneal injection of Thiopental in the dose as described above and burned by 20 s exposure to the hot water (90° ± 2° C) of the shaved dorsal skin. This produced a full-thickness burn of 30% total body surface (17). The 3rd group were the animals pretreated with Thiopental in the same dose as described previously and burned as above which were treated intraperitoneally with the highly cleaned extract of calf thymus (TFX POLFA), in the dose of 9 mg/kg per body weight one hour after burn.

The level of lipid peroxidation products in all three groups was investigated. Conjugated dienes (CD), lipid hydroperoxides (HPETE) and malonyldialdehyde (MDA) were measured in the plasma 24 and 48 h after burn injury. Investigations were made immediately, 24 and 48 h after start of the experiment also on the control rats. The levels of lipid peroxidation products were examined in plasma obtained from all three groups of anaesthetised rats at the time as described above and then prepared in the adequate way for different methods described below.

Conjugated dienes were measured according to the iodometric assay described by Buege and Aust (3) in Ward’s modification (29). CD was extracted from plasma using 2:1 (vol/vol) mixture of chloroform and methanol. Then, 7 ml of the chloroform-methanol mixture, preheated to 45°C, was added to 0.5 ml of plasma. The mixture was then vigorously mixed for 2 min and then centrifuged at 1500 g for 5 min. Five ml from the lower layer (chloroform) was aspirated and mixed with 2.0 ml of distilled water acidified with 0.1 N HCl to pH of 2.5. The mixture was then mixed and centrifuged (as described above), and 2.0 ml of the lower layer was aspirated and then dried under a flow of nitrogen gas. The residue was reconstituted with 0.5 ml of n-heptane and measured spectrophotometrically at 233 nm against n-heptane. The
concentration of CD investigated samples tissues was measured in OD (Optical Density) per 1 ml of plasma.

Lipid hydroperoxides were measured according to the iodometric assay described by Buege and Aust (3) in Ward’s modification (29). One ml of plasma was mixed with 7.0 ml of chloroform-methanol (2:1, vol/vol), agitated for 2 min and then subjected to centrifugation (1500 g) for 5 min. Then, 5 ml of the lower chloroform layer was aspirated and dried under nitrogen. When dry, 1.0 ml of acetic acid-chloroform mixture (3:2, vol/vol) and then 0.05 ml of potassium iodide (1.2 g/vol) were added and this mixture was rapidly shaken and shielded from light for 5 min. Then 3.0 ml of cadmium acetate (0.5 g%) was added, the solution was shaken vigorously and cleared by centrifugation. The upper (water) phase was aspirated and its absorbance at 352 nm was determined against 0.5% water solution of cadmium acetate.

Concentration of malonyldialdehyde was measured in plasma according to Ledwożyw method (19). Malonyldialdehyde was estimated by the thiobarbituric acid reactions as follows (36): 0.5 ml of plasma was mixed with 2.5 ml of 1.22 mol/l trichloroacetic acid in 0.6 mol/l HCl and allowed to stand for 15 min. Then, 1.5 ml of thiobarbituric acid (TBA) solution was added (TBA solution was obtained by dissolving 500 mg TBA in 6 ml 1 mol/l NaOH and then adding 69 ml H2O) and thereafter heating for 30 min in a boiling water bath. Then the mixture was cooled to room temperature. 4 ml of n-butanol was added to it and the obtained mixture was vigorously shaken for 3 min and centrifuged 10 min at 1500 g. After that, the organic layer was removed and its absorbance at 532 nm against n-butanol was measured. The concentration of MDA in the samples was determined from the standard curve plotted by using malondialdehyde bis-dimethylacetal. Finally, the concentration of MDA was described in nmol/0.5 ml of plasma.

The protein in plasma was determined according to Lowry et al. (20).

Statistical analysis of differences was performed according to the Student's t-test for unpaired data and the non-parametric Mann-Whitney U test.

### Results

**Quantity of CD (Fig. 1).** The concentration of CD in the plasma of the control rats amounted mainly 0.175 OD per ml in every time of examination. The level of CD in plasma after burn was significantly higher than that in control animals, mainly 0.344. Maximum values were assessed 24 h after burn, and they dropped slightly after 48 h. TFX administration caused the decrease in CD concentration in plasma in all experimental cases. After 24 h post burn, the radical decrease in CD plasma concentration was observed, which was almost on the same level 48 h after burn. These values are statistically significant.

**Quantity of HPETE (Fig. 2).** The concentration of HPETE in plasma of the control rats amounted to 0.13 OD per ml. In the burned rats the means were much higher than in the control ones as early as in 24 h after burn, and dropped rapidly in 48 h after burn, but remained still higher than in control. In the animals treated with TFX, HPETE concentration was increasing constantly in 24 h after burn as well as in 48 h, when it reached main value 4.4 OD per ml of plasma. Each time the measurements were greater than in the control and burned groups. These values are statistically significant.
**Quantity of MDA (Fig. 3).** The concentration of MDA in plasma of the burned rats increased significantly in relation to the TFX treated, as well as to the control group in all investigated periods. In the TFX treated group, the concentration of MDA was decreased at the 24th h after injury in comparison to the burned rats and was higher than in the control, and its concentration at the 48th h was higher than in the previous period. TFX treatment decreased the level of plasma MDA below that in the burned group at the 24th and 48th h after injury, but this decrease was not proportional to the burned group values and these items are statistically significant. TFX treatment markedly decreased concentration of CD and MDA, what was statistically significant. Only the HPETE showed the proportionally increased generation in comparison to burn group only in 24 h of the experiment, but it was not so spectacular in 48 h. These data are statistically significant.

In cases of TFX treatment of burn rats, the concentration of MDA was significantly decreased, and the best results were at the 24th h after burn. At the 48th h it was paradoxically greater than in the previous period, whereas the concentration of MDA in only burned rats was diminished. It looks as if a self-generation of MDA after TFX treatment of burned rats was time dependent.

![Fig.1](image_url)

**Fig.1.** Effect of thymus extract (TFX) on the level of conjugated dienes (CD) in the plasma after thermal injury in rats. The data are expressed as mean (± SD).

* Significant difference (P<0.01) vs. control group, # significant difference (P<0.005) vs. burn group.
Fig. 2. Effect of thymus extract (TFX) on the plasma hydroperoxides (HPETE) concentration after thermal injury in rats. The data are expressed as mean (± SD). * Significant difference (P<0.01) vs. control group, # significant difference (P<0.01) vs. burn group.

Fig. 3. Effect of thymus extract (TFX) on the level of malonyldialdehyde (MDA) in the plasma after thermal injury in rats. The data are expressed as mean (± SD). * Significant difference (P<0.01) vs. control group, # significant difference (P<0.01) vs. burn group.
Discussion

Thermal injury initiates systemic inflammatory reactions producing burn toxins, an inflammatory response, oxygen radicals, and finally peroxidation. The obtained results showed that lipid peroxidation process began immediately after severe burn and was continued during the whole 48 h post burn period. Huo et al. (14) investigating lipid peroxidation induced by human burn skin reported that plasma lipoperoxide concentration measured as malondialdehyde increased in the first few hours post burn. Demling and LaLonde (9) reported the increase in MDA and CD levels in plasma at the 3rd h after burn and the high concentrations of lipids peroxidation products persisting into the 5th d after thermal injury. Pintaudi et al. (25) reported markedly increased plasma levels of MDA according to the extent of injury, however, the plasma level of CD and HPETE was only slightly higher than in controls at the baseline, followed by a rapidly progressive decrease, indicating a massive loss of circulating lipids by the acute thermal injury. Hilton (13) postulated that the products of metabolism of polyunsaturated fatty acids (PUFA) were the substances causing the plasma volume loss. The two major pathways for PUFA are involved in this process – cyclooxygenase transformation to form prostaglandins and lipoxygenase transformation to form fatty acid hydroperoxides. Our investigation showed that TFX administration caused the decrease in plasma CD concentration. In the animals treated with TFX, HPETE concentration in plasma increased constantly in 24 h after burn as well as in 48 h. TFX treatment decreased the levels of plasma MDA below the burned at the 24th and 48th h after injury, but this decrease was not proportional to the burn. The existing high level of CD shows that the lipid peroxidation process was initiated. These forms may be converted to MDA by cyclooxygenase pathway or to HPETE and in consequence to leukotrienes. This is possible due to the lipoxygenase activity which is partially dependent on lymphocyte proliferation (28). The arachidonic acid (AA) metabolites play the major role in lymphocyte activity regulation. Wood et al. (30) reported that failure to produce IL-2, a powerful mediator of cellular immune responses, is an important mechanism underlying the defective cell mediated immunity seen in burned patient. Kowal-Vern et al. (17) observed the increase in IL-6 and IL-2 levels in proportion to the severity of the burn wound and its size. Cyclooxygenase inhibitors enhanced the IL-2 production. On the other hand, TFX added to lymphocyte enhanced IL-2 generation which has an influence on the early molecular events (23). The ability of thymus hormones (5-thymosine and TFX) to promote the lymphokine generation, with addition of antioxidants, diminished lipoxygenation. Depleted levels of these natural antioxidants following burn injury may cause paradoxically enhanced HPETE generation, which was observed in our investigation. Neutrophils diffusely invade the organs and burned skin following burn injury. Thus, the reducing in systemic neutrophilic injury and related lipid peroxidation in burn may have a benefit effect (4).

Aldehydic products of lipid peroxidation act as endotoxins, causing damage to various tissues and organs (25). Damage to the liver (4) was assessed by increased plasma levels of aspartate and alanine transaminases within 7-15 d after burn. Saitoh et al. (26, 27) noticed that the severe burn causes the increase in the level of MDA in the plasma, lung, and kidney of injured rats. Enhanced antioxidant enzymes activities are the response of the organism to the oxidation shock (14). Some authors (12) reported that lipid peroxidation and free radicals play the major role as secondary mediators in the evolution of a burn shock. The biological inhibitor of toxic oligopeptides infused
intravenously in rats caused the acceleration of the epithelization of burn wound and was able to lower the levels of molecular products of lipid peroxidation (14).

Demling and Lalonde (7, 6) reported the increase in MDA and CD levels in plasma at 3rd h after burn and the high concentration of lipids peroxidation products lasting 5 d after burn. L’vovskaja et al. (21) demonstrated the acceleration of the epithelization of the burn wound in conditions of lower levels of molecular products of lipid peroxidation.

In spite of the increase in total antioxidant status (TAS) in plasma value and enhanced activity of enzymes, lipid peroxidation process is intensive after severe burn and so the antioxidant defense is unsuccessful. In this case, research relative to antioxidants treatment and TFX treatment can bring the hope to change the adversity of burned patients. TFX promote the enhanced elimination of infection factors and diminish tissue pathology via increased local and general immune response.

References