INFLUENCE OF EARLY EXCISION OF BURN WOUND ON THE LIPID PEROXIDATION IN SELECTED RAT TISSUES

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Lipid peroxidation products in burn and after early excision of burn wound were assessed. Obtained results show 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 in the heart, liver and kidney, and slightly enhanced in the lung. Our investigations suggest that early excision diminished the CD generation, which is markedly expressed in the case of the heart and liver and not significant in lung. Early excision prevented the ongoing post burn peroxidation in distant organs. It seems to be of great prognostic importance in severe burn injury.

Key words: rats, burn, early excision, lipid peroxidation, lung, kidney, heart, liver.

Reactive oxygen forms (ROF) or free radicals are the atoms or molecules containing unpaired electrons on the last shell, so they are highly reactive and responsible for oxidation damage of lipids (1, 21). Free radicals as well as histamine and prostaglandins released from the burn wound cause lipid peroxidation in the skin. Peroxidation of lipid forming cell membranes causes changes in their structure and, in turn, the dysfunction of affected cells. In consequence they cause burn edema, intravascular haemolysis, hypovolemic shock and adult respiratory distress syndrome or multiple organ failure syndrome (2, 4, 13). Lipid peroxidation process occurs after severe burn and is continued during the whole 48 h of the post burn period.

One of the toxic forms of oxygen-free radicals are particularly toxic for cells. One of the most vital biological processes associated with the activity of free radicals is the peroxidation of lipids, which are particularly numerous in phospholipids of cell membranes. These processes can damage the lipid part of the biological membrane, which leads to the loss of its function (5) 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 index of free radical activity.

One of the causative agents responsible for the development of burn shock and distant organ injury in animal models of burn trauma are oxygen radicals. The distant organs involved in this thermal injury during experimental skin burn are: the lungs, heart, liver, kidney and gastrointestinal mucosa. In all cases of this trauma neutrophils diffusely invade these organs being the potent source of reactive oxygen metabolites. The aim of this study was to estimate the effect of early excision of burn wound
(obtained in consequence of severe burn injury) on the lipid peroxidation intensity in lung tissue, heart tissue, kidney and liver.

**Material and Methods**

The study was performed on male Wistar rats with body weight 250 g ± 50 g. They were maintained 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 second group were rats with third-degree burn of 30% of the body surface area. Animals were anesthetized and burned by 20 s exposure to hot water (90º ± 2ºC) of the shaved dorsal skin. This produced a full-thickness burn of 30% total body surface area. The 3rd group were the animals pretreated with Thiopental in the same dose as described previously and burned as above, which underwent early excision of burn wound. The early excision was performed according to Jethon (14).

The level of lipid peroxidation products in liver, kidney, heart and lung tissues in all three groups were investigated. Conjugate dienes (CD), lipid hydroperoxides (HPETE) and malondialdehyde (MDA) were estimated. The levels of estimated values in the early excision of burn wound group were measured time-dependently (2, 4, 6, 12, 24 h after burn).

**Preparation of tissues.** Investigations were made immediately after getting the tissues, which were obtained at 2, 6, 12, 24 and 48 h after burn injury and from control rats. The levels of lipid peroxidation products were examined in tissues obtained from all three groups of anaesthetized rats at the time as described above and then prepared in the adequate way for different methods described below.

**Assay of Conjugated Dienes (CD).** Conjugated dienes were measured according to the iodometric assay described by Buege and Aust (3) in Ward's modification (28). Tissues were extracted with 0.8 ml of distilled water being added for each 1.0 g of wet tissue. The material was then homogenized and subsequently, for 1 g of tissue wet weight, 6.0 ml of chloroform-methanol (1:2 vol./vol.) was added. This mixture was vigorously shaken, then mixed with 2 ml of HCl (prepared like above) per 1 g of wet tissue. Finally, the material was cleared by centrifugation, and the chloroform layer was aspirated and treated as described above. The concentration of CD in investigated tissues was marked in OD (Optical Density) per 1 g of tissue.

**Assay of Lipid Hydroperoxides (HPETE).** Lipid hydroperoxides were measured according to the iodometric assay described by Buege and Aust (3) in Ward’s modification (28). One gram of tissue was washed with 0.9% NaCl solution and then homogenized in 5 volume parts of 0.2 M phosphate buffer (pH=7.4) using glass homogenizer. Homogenate of 1 g of lung tissue 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.
Assay of Malondialdehyde (MDA). Concentration of MDA was measured in homogenate of examined tissue according to Ledwożyw’s method (17). The tissue was washed with 0.9% NaCl solution, homogenized in 5 volume parts of 200 nM phosphate buffer (pH=7.4) using glass homogenizer and then the sample was centrifuged at 2000 g for 15 min to obtain the homogenate supernatant. Malondialdehyde was estimated by the thiobarbituric acid reactions as follows (36): 0.5 ml of plasma or tissue homogenate supernatant was mixed with 2.5 ml 1.22 mol/l trichloroacetic acid in 0.6 mol/l HCl and allowed to stand for 15 min. Then 1.5 ml thiobarbituric acid (TBA) solution was added (TBA solution was obtained by dissolving 500 mg of 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 and 4 ml of n-butanol was added to it and the 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-methyloacethal. Finally, the concentration of MDA was described in nM/mg wet tissue. The protein in tissues homogenates was determined by the method of Lowry et al. (18).

Statistical analysis. Lipidperoxidation endproducts are presented as mean ±SD and differences between groups were analyzed by Student’s t test. A statistically significant difference was accepted if P<0.05.

Results

Quantity of CD in investigated organs (Figs 1-4). The concentration of CD in the heart, lung, liver and kidney of the control rats amounted to 0.100 OD/g tissue, 0.77 OD/g tissue, 0.100 OD/g and 0.160 OD/g tissue, respectively, at every time of examination. The level of CD in the tissues after burn was significantly higher than in control animals, by 120 to 330%. Maximum values were assessed at the 4th and 6th h after burn. Early excision caused the decrease in CD concentration levels in almost all experimental cases. Severe burn also caused the increase in the CD quantity in the lung tissue but the level of CD measured at the 2nd and 4th h after injury in early excision increase in comparison with control and burned animals from 116.8% of control level at the 2nd h to 142.8% of control level at the 48th h and 112.5%-110.0% of only burned, respectively. These values were statistically significant. After 48 h the values were almost the same in burned and early excised group. The similar results were obtained at the 2nd h of experiment in the kidney. Early excision caused markedly decreased concentration in CD (besides the lung case), whereas it was statistically significant only at the 4th, 6th and 12 h after burn. Only the kidney shows the proportionally decreased CD generation in comparison to the burn only group in the whole time of experiment, but not all obtained data were statistically significant.

Quantity of HPETE in investigated organs. (Figs 5-8). The concentration of HPETE in tissues of the control rats amounted in the heart 0.028 OD/g, in the lung 0.021 OD/g, in the liver 0.018 OD/g and in the kidney 0.035 OD/g. After burn the means were higher than the control in the heart, lung, liver and in kidney, as early as at the 2nd h. At the 4th h after burn the concentration of HPETE achieved maximum value in all tissues. Then they decreased constantly but in every time the measurements were greater than in the control group. The concentration of HPETE in the lung tissue
was significantly decreased at the 2nd and 4th h after excision, which has no effect in the other periods of investigations. Early excision decreased slightly the value of HPETE in liver tissues to the end of the second day after burn injury. In the kidney, after slightly increase in HPETE concentration at the 2nd h, early excision caused massive decrease of this value at the 4th h (about 71.0% in comparison to the burn) and significantly decreased these values in all the later time periods. In heart tissue excision enhanced the HPETE generation at the 2nd h and significantly decreased it at the 4th h after burn injury. These values are statistically significant.

**Quantity of MDA in investigated organs.** (Figs 9 - 12). The concentrations in MDA in tissues of the burned rats increased significantly in relation to the control group in all investigated organs and amounted appropriately in the heart to about 105.2% at the 2nd h, 195% at the 6th h, 292.2% at the 12th h, 167.9% at the 24th h and at the 48th h 125.3% of the control value. In the lung tissue the concentration of MDA was increased as early as in 2 h after injury (116.9% of control) and the highest concentration was noticed at the 4th h when amounted to about 177.7% of the control. In the liver tissues the concentration of MDA was increased in burned animals at the 2nd h after injury (136.2% of control) and achieved a maximum concentration at the 6th h when amounted about 163.1% of the control value. Early excision decreased these levels below the control at the 2nd, 12th and 48th h after injury, but these items were statistically not significant. In the kidney tissues obtained from burned rats the concentration of MDA increased rapidly and maximally just 4 h after injury (279% of control) and the high concentration was noticed in the whole following period of investigation. These data were statistically significant.

In all cases of early excision of burn wound the concentration of MDA was significantly decreased, whereas the best results were in heart tissue, in which the average concentration after 2, 4, 7, 12 and 48 h was similar to the control group. In liver and kidney tissues the concentration of MDA measured after early excision dropped below the average of control at the 2nd h and achieved maximum at the 4th h after burn (about 163% and 148.1% of control, respectively) and proportionally decreased to about control level in 48 h. In lung tissue early excision has no significant role in MDA concentration, much better this procedure caused the increase in the malonyldialdehyde concentration at the 2nd and 12th h after excision (in comparison to burned animals lung tissue). These items were not significant.

![Fig. 1. Heart tissue CD concentration in different groups of rats. The data are expressed as mean ± SD (*) significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group.](image)
Fig. 2. Lung tissue CD concentration in different groups of rats. The data are expressed as mean ± SD (* significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group).

Fig. 3. Liver tissue CD concentration in different groups of rats. The data are expressed as mean ± SD (* significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group).

Fig. 4. Kidney tissue CD concentration in different groups of rats. The data are expressed as mean ± SD (* significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group).
Fig. 5. Heart tissue HPETE concentration in different groups of rats. The data are expressed as mean ± SD (* significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group).

Fig. 6. Lung tissue HPETE concentration in different groups of rats. The data are expressed as mean ± SD (* significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group).

Fig. 7. Liver tissue HPETE concentration in different groups of rats. The data are expressed as mean ± SD (* significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group).
Fig. 8. Kidney tissue HPETE concentration in different groups of rats. The data are expressed as mean ± SD (* significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group).

Fig. 9. Heart tissue MDA concentration in different groups of rats. The data are expressed as mean ± SD (* significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group).

Fig. 10. Lung tissue MDA concentration in different groups of rats. The data are expressed as mean ± SD (* significant difference (P<0.05) vs. control group # significant difference (P<0.05) vs. necrectomy group).
Discussion

Thermal injury initiates systemic inflammatory reactions producing burn toxins, an inflammatory response, oxygen radicals and finally peroxidation. The relationships between the amount of products of oxidative metabolism and natural scavengers of free radicals determine the outcome of local and distant tissue damage, and further organ failure in burn injury. The obtained results show that lipid peroxidation process begins immediately after severe burn and is continued during the whole 48 h post burn period. Burn enhanced the lipid peroxidation products in the heart, the liver and the kidney, slightly enhanced in the lung. Huo et al. (12)
investigating lipid peroxidation induced by human burn skin reported that plasma lipoperoxides concentration measured as malondialdehyde increased in the first few hours post burn. Demling and LaLonde (7) reported the increase in MDA and CD levels in plasma at the 3rd h after burn and at the high concentrations of lipids peroxidation products persisting into the 5th d after thermal injury. The intensification of lipid peroxidation process in the lung can be in part a consequence of the early sequestration of neutrophils and macrophages in lung tissues (7). Till et al. (25) noticed that systemic treatment of burned animals by catalase CAT and superoxide dismutase SOD are the protection from lung injury after burn. The intensive lipid peroxidation in the lung depends on the enhanced activity of xantine oxidase, which dramatically increased after burn injury (10, 26). Demling et al. (5, 6, 7) reported that lipid peroxidation is not increased in large airway or lung parenchyma early after smoke exposure. The addition of burn significantly increases lung parenchymal lipid peroxidation, but the oxidant changes do not correspond with the degree of early lung dysfunction.

Pintaudi et al. (22) 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 control at the baseline, followed by a rapidly progressive decrease, indicating a massive loss of circulating lipids caused by the acute thermal injury. Saitoh et al. (23) noticed that severe burn causes the increase in the level of MDA in the plasma, the lung, and the kidney of injured rats. Enhanced antioxidant enzymes activities are the prevention and response of organism to the oxidation shock (15). The remarkable decrease in superoxide dismutase and TAS (total antioxidating status) observed in burn injury caused the unbalance in the lipid peroxidation-antioxidation system (4).

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 oligopetides infused intravenously in rats causes the acceleration of the epithelization of burn wound and is able to lower the levels of molecular products of lipid peroxidation (15).

Pintaudi et al. (22) reported that aldehydic products of lipid peroxidation act as endotoxins, causing damage to various tissue organs. Damages to the liver were assessed by increased plasma levels of aspartate and alanine transaminases within 7-15 d after burn. They also mention the decrease of lipid antioxidant in accordance with severity of the injury which is also reflected as a systemic oxidant stress (11). Chinese findings suggest that early necrectomy is important to prevent adhesion molecule expression and SIRS (12). They investigated lipid peroxidation induced by human burn skin and reported that plasma lipoperoxides concentration measured as malondialdehyde increased in first few hours post burn. Demling and Lalonde (7) reported the increase in MDA and CD levels in plasma at 3 h after burn and the high concentration of lipids peroxidation products occurring until the 5th d after burn.

Our investigation suggest that early excision diminished the CD generation which is markedly expressed in the case of the heart and liver and not significant in the lung. Early excision prevented the ongoing post burn peroxidation in distant organs and lung inflammation seen 3 d after burn. Those clinical observations suggest that it may be a consequence of decreased lipid peroxidation observed in our investigation as early as on the 2nd d after burn.

L’vovskaja et al. (15) studied the acceleration of the epithelization of the burn wound under conditions of lower levels of molecular products of lipid peroxidation.
In spite of the increase of TAS value and enhanced activity of enzymes, lipid peroxidation process is intensive after severe burn so the antioxidant defense is unsuccessful. In this case research relative to antioxidants treatment and early excision of burn wound can bring the hope of the change of adversity of burned patients.

References