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# LIPID PEROXIDATION IN THE KIDNEYS OF RATS WITH NEPHRITIS CAUSED BY NEPHROTOXIC SERUM AND WITH PROTEINURIA INDUCED BY ALBUMIN LOADING

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**KEY WORDS:** nephrotoxic nephritis; lipid peroxidation; proteinuria; albumin loading

Evidence has now been obtained to demonstrate the important role of free oxygen radicals (FOR) in the pathogenesis of some experimental models of immune damage to the kidneys: nephrotoxic nephritis (NTN) [11], Heymann's passive nephritis [12], and rejection of a transplanted kidney [10]. It has been shown that FOR can be produced by both polymorphs and monocytes infiltrating the glomerulus at different stages of immune damage [7, 11] and by activated mesangial cells of the glomerulus [5]. One of the main mechanisms of realization of the damaging action of FOR is their ability to initiate lipid peroxidation (LPO) of cell membranes.

It was accordingly decided to investigate LPO in kidney tissue in one form of immune injury, namely NTN induced by antirenal serum. Since NTN is characterized by considerable proteinuria, to assess its effect on LPO processes in the kidneys their activity was determined in rats with proteinuria induced by a short but massive load of exogenous protein, when no visible immune reactions can be found in the kidney tissue [9].

## EXPERIMENTAL METHOD

Experiments were carried out on 61 male Wistar rats weighing initially 150-200 g. Of the total number of rats 20 had NTN, nine had proteinuria induced by injection of large quantities of protein, and 32 rats served as the control. NTN was induced by injecting nephrotoxic serum (NTS) into the femoral vein in a dose of 0.8 ml/100 g body weight on 1 or 2 consecutive days. NTS was obtained by immunizing a rabbit with the glomeruli of a rat kidney [3]. Protein loading and consequent proteinuria were produced by intraperitoneal injection of human albumin, dissolved in physiological saline, in a dose of 1.5-2 g protein per rat weighing 200 g, on 3 consecutive days [9].

In the experiments of series I activity of LPO processes was studied in the kidneys at different stages of development of NTN: 30 min and 3 h after a single injection of NTS (six and three rats respectively), and on the 4th and 16th days after the first of two injections of NTS (six and five rats respectively). The control consisted of 25 rats receiving normal rabbit serum instead of NTS.

In the experiments of series II LPO activity was determined in the kidneys of nine rats after three daily injections of protein, and in seven control rats receiving physiological saline intraperitoneally.

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TABLE 1. Analysis of LPO in Renal Cortex of Rats with NTN and with Albumin Loading and Biochemical Characteristics of these Models (M ± m)

| Parameter | Nephrotoxic nephritis | | | | | | Albumin loading | |
|---|---|---|---|---|---|---|---|---|
| | 30 min | | 4 days | | 16 days | | control (7) | experiment (9) |
| | control (6) | experiment (6) | control (7) | experiment (6) | control (9) | experiment (5) | | |
| Body weight, g | 137±4,0 | 129±3,8 | 133±3,9 | 130±3,8 | 260±7,5 | 241±7,6 | 225±17 | 209±5,3 |
| MDA level, μmoles/g | | | | | | | | |
| initial | 0,033±0,01 | 0,036±0,01 | 0,025±0,006 | 0,035±0,01 | 0,036±0,01 | 0,02±0,005 | 0,026±0,005 | 0,03±0,01 |
| after incubation for | | | | | | | | |
| 15 min | 0,69±0,08 | 0,62±0,21 | 0,69±0,1 | 0,42±0,16 | — | — | 0,94±0,04 | 0,51±0,08* |
| 30 min | 1,52±0,04 | 1,41±0,2 | 1,58±0,1 | 1,04±0,25 | 1,31±0,05 | 0,94±0,12*** | 1,61±0,04 | 1,30±0,05* |
| 45 min | 1,85±0,06 | 1,96±0,11 | 1,88±0,1 | 1,36±0,19*** | 1,62±0,05 | 1,19±0,08** | 1,82±0,03 | 1,54±0,05* |
| Proteinuria, mg/18 h | — | — | 3,13±0,46 | 107±27,5* | 3,13±0,5 | 199±24,5* | 3,13±0,5 | 294±52* |
| Blood serum: | | | | | | | | |
| total protein, g/liter | — | — | 66,0±1,5 | 53,0±2,6* | 66,5±1,2 | 50,1±1,9* | 68,0±0,6 | 81,7±1,7* |
| total cholesterol, mmoles/liter | — | — | 2,12±0,12 | 3,8±0,48** | 2,15±0,13 | 7,6±0,78* | — | — |

**Legend.** Number of animals given in parentheses. *$p < 0.001$, **$p < 0.01$, ***$p < 0.05$ compared with corresponding control.

In the rats of both series, on the eve of the investigation of kidney tissue protein excretion with the urine in 18 h was determined by the sulfosalicylate method. The total protein content was determined in blood serum obtained from the tail by the biuret method, and the total cholesterol was determined by Ikles' method [1].

Activity of LPO processes was studied in homogenate of the renal cortex. The kidneys were removed under hexobarbital anesthesia and washed with cold physiological saline. A homogenate of the cortical layer was prepared in phosphate buffer (pH 5.9) in the proportion of 10 mg tissue to 1 ml buffer. The homogenate was incubated in thermostated cells (37°C) with constant mixing and aeration for 60-70 min. LPO was initiated with ascorbic acid ($10^{-5}$ M). Samples for determination of the LPO end product, malonic dialdehyde (MDA), were obtained before addition of ascorbate (initial level) and after incubation for 15, 30, and 45 min. In the series of experiments with NTN and in series II the kinetic method of investigation of LPO was used, so that it was necessary to take additional samples of homogenate after incubation for 5, 10, 20, 60, and 70 min, and the results of MDA determinations were presented as kinetic curves. They were used to calculate the maximal rate of MDA production in the homogenate, and the induction period $\tau$ was found, by dropping a perpendicular on to the time axis from the point on the graph after which the MDA concentration began to rise in a straight line [2]. The MDA concentration in the samples of homogenate was determined by its reaction with 2-thiobarbituric acid [6] and was expressed in micromoles or nanomoles per gram wet weight of tissue.

Proteinuria was characterized by electrophoresis of urinary proteins in a linear polyacrylamide gel gradient (4-30%) [4].

## EXPERIMENTAL RESULTS

The NTS used in the study was found to have sufficient activity to induce the development of a nephrotic syndrome (considerable proteinuria, hypoproteinemia, hypercholesterolemia) in the rats on the 4th day after its first injection, and its manifestations increased in severity until the 16th day of the disease (Table 1).

Activity of LPO in the kidney tissue was studied starting with the earliest times of development of NTN. An increase in permeability of the basement membranes of the glomeruli and increased passage of protein molecules are known to take place as early as 30 min after injection of active NTS [8]. After 2-3 h the proteinuria increases, due, it is supposed [11] to infiltration of the glomeruli by polymorphs, which, being activated by the antigen–antibody complex, produce FOR, which can damage the basement membranes as a result of initiation of LPO processes.

However, 30 min (Table 1) and 3 h (Fig. 1a) after injection of NTS, no activation of LPO could be found in the kidneys: the original levels of MDA and its production in response to stimulation of LPO by ascorbate did not differ from the control values.

On the 4th day of the disease activity of ascorbate-induced LPO in homogenates of the renal cortex was lower than in the control: MDA production during incubation for 15 and 30 min had a tendency to decrease and after incubation for 45 min it was significantly lower.

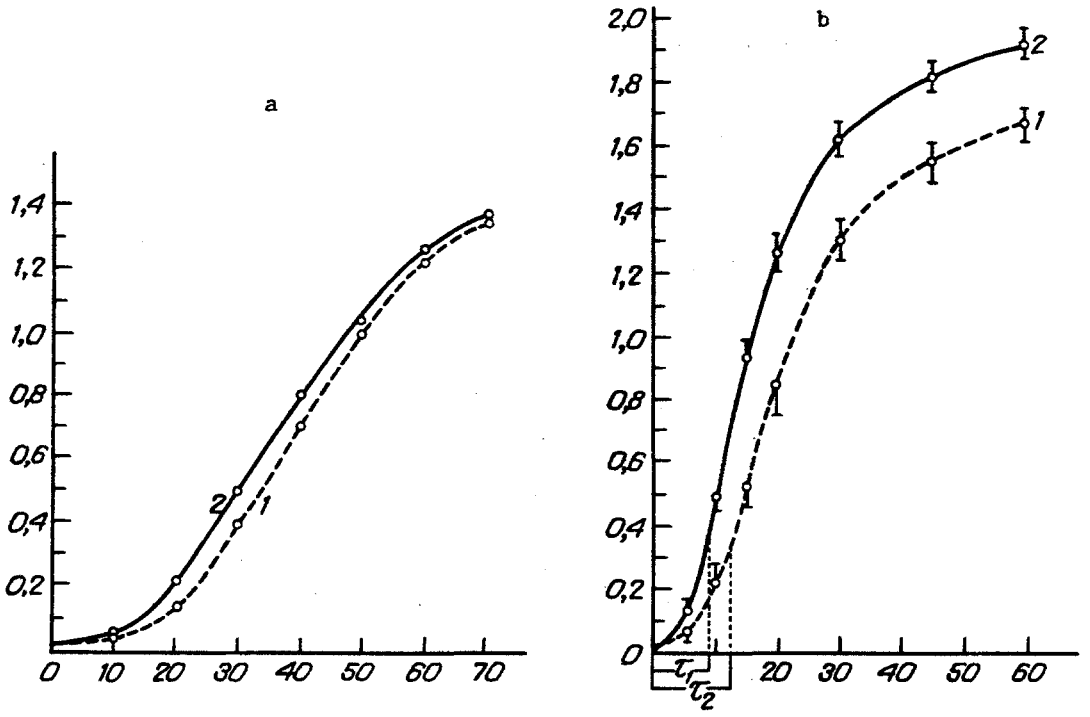


Fig. 1. Kinetic curves of ascorbate-induced LPO in homogenates of rat renal cortex. a) 3 h after injection of NTS (1) or normal rabbit serum (2), b) after administration of albumin (1) or physiological saline (2) intraperitoneally for 3 days. Abscissa, incubation time (in min); $\tau_1$ and $\tau_2$) periods of induction (in min); ordinate, MDA concentration (in $\mu$moles/g wet weight of tissue).

Day 16 of the disease corresponded to the second, autologous phase of NTN, when the kidney tissue was subjected to repeated attack by antibodies, now being produced actually in the rat's own body to rabbit antirenal antibodies fixed in the kidneys. Investigations at this time (Table 1) revealed less liability of the kidney tissue of rats with NTN to develop ascorbate-dependent LPO compared with the control, after incubation of the homogenates for either 30 min or 45 min. In our view, the factor inhibiting LPO may be the development of proteinuria, with the entry of plasma proteins possessing antioxidative properties, such as transferrin and ceruloplasmin [13], into the mesangium of the glomerulus and other parts of the nephron.

To test this hypothesis, we studied LPO processes in the kidneys of rats with marked proteinuria, developing in response to loading with human albumin. In fact, we found weakening of activity of ascorbate-induced LPO at all times of incubation of the kidney tissue (Fig. 1b; Table 1). The maximal rate of MDA production was 65 ± 3.9 nmoles/g/min in the experimental group and 83.1 ± 2.3 nmoles/g/min in the control group ($p < 0.001$). Correlation analysis revealed significant negative correlation between MDA production during the first 15 min of incubation of the renal homogenate and the severity of the proteinuria ($r = -0.83$, $p < 0.01$). On analysis of the kinetic curves of ascorbate-dependent LPO in the kidneys (Fig. 2) significant lengthening of the induction period was found in rats with proteinuria induced by protein loading compared with the control ($\tau = 11.9 \pm 1.0$ and $8.4 \pm 0.3$ min respectively, $p < 0.01$), evidence in support of the greater supply of antioxidants available for the kidneys of the rats with proteinuria [2].

Electrophoresis of the urinary proteins of rats with NTN and with protein loading (Fig. 2) revealed predominant excretion of albumin and proteins with lower molecular mass. However, electrophoresis clearly demonstrated the presence of proteins with molecular mass of the order of 80-160 kDa, or even 230 kDa in the urine, i.e., in the models used, the entry of protein "quenchers" of LPO, transferrin, and ceruloplasmin, with molecular masses of 88 and 132 kilodaltons respectively, and their passage along the nephron, cannot be ruled out.

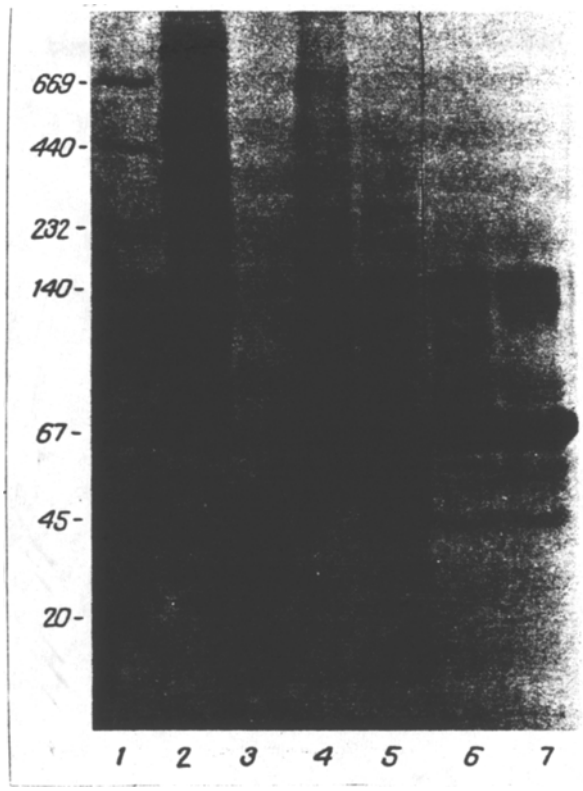


Fig. 2. Electrophoresis of urinary proteins in a linear polyacrylamide gel gradient. 1) Reference substances, 2) blood serum of intact rat, 3) urine of intact rat, 4, 5) urine of rats with NTN on 16th day of disease, 6, 7) urine of rats after albumin loading for 3 days. Ordinate, molecular mass of proteins (in kilodaltons).

We thus found no evidence of intensification of ascorbate-induced LPO in the renal cortex of rats with NTN in the early stages of its development (0.5 and 3 h). A tendency was noted for LPO activity to be reduced with the formation of a nephrotic syndrome (the 4th day of NTN) and inhibition of LPO when the pattern of the disease was fully developed (16th day). The experiments with proteinuria induced by protein loading and analysis of the uroproteins suggest that proteinuria has an inhibitory effect on LPO in the kidneys, possibly through excretion of proteins possessing antioxidative properties.

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