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# Direct Pharmacological Correction of Oxidative Stress in Rat Kidneys Does Not Facilitate Diabetic Nephropathy

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## Abstract

***The aim*** of this study was to evaluate the effect of alpha-tocopherol acetate (ATA) on the activity of free-radical oxidation (FRO) in renal tissue and renal function in rats with experimental streptozotocin (STZ)-induced diabetes mellitus (DM).

***Methods and Results*:** Experiments were conducted on 22 male Wistar rats aged 60-100 days and weighing 250-300 g. The animals were divided into two groups: Group 1 (control) and Group 2 (experimental). To induce DM, the animals were injected intraperitoneally 1ml of STZ solution in the citrate buffer at a dose of 65 mg/kg. For more selective modeling of type 2 DM, the rats were previously injected with an intraperitoneal solution of cytoflavin based on a nicotinamide dose of 115 mg/kg. In Croup 2, ATA was administered in the period from the fifth to eighth weeks, inclusive, intragastrically through a tube at a daily dose of 300 mg/kg.

Experiments showed that after a 4-week course of ATA, the concentration of thiobarbiturate-reactive products in the kidney tissues of the rats in Group 2 was 5.3 times lower than in Group 1. The activity of all antioxidant enzymes did not differ between the two groups. In both groups, during all 8 weeks of the experiment, the levels of renal excretion of glucose, protein, and creatinine significantly exceeded the initial level, while the level of diuresis remained stable.

***Conclusion***: The long-term administration of ATA in experimental streptozotocin (STZ)-induced DM is accompanied by a significant suppression of the activity of the FRO processes in the kidneys, but does not lead to an improvement in the course of diabetic nephropathy. (**International Journal of Biomedicine. 2021;11(3):296-300.**)

**Key Words**: kidneys • diabetic nephropathy • tocopherol acetate • oxidative stress

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## Abbreviations

**ATA,** alpha-tocopherol acetate; **CAT**, catalase; **DM**, diabetes mellitus; **DN**, diabetic nephropathy; **FRO,** free-radical oxidation; **GPx**, glutathione peroxidise; **OS**, oxidative stress; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase, **TBRP**, thiobarbiturate-reactive products.

## Introduction

Numerous studies have proposed that OS plays a crucial role in the progression and severity of diabetic nephropathy (DN).[1-3] The activation of FRO in the kidneys, against the background of diabetes mellitus (DM), has been shown in many works, including our previous study.[4] However, the mechanisms that develop oxidative damage to the renal glomerulus are quite diverse. They can be direct and indirect. It is generally accepted that in DM, glucose and its metabolites in urine can directly suppress the activity of cellular antioxidants, such as glutathione.[5-8] Numerous studies have shown that in DM, there exists an accumulation of advanced glycated end-products,[5,9,10] increased OS,[11] and enhanced angiotensin II levels.[5,12] The adverse effects of most of those factors have often been linked to the generation of ROS.[3,13] Many sources

of ROS contribute to increased OS; however, NADPH oxidases (Nox) and their catalytic subunit are the only known enzyme family solely dedicated to producing ROS.[3,5,13-15] Furthermore, Nox isoforms are upregulated in the presence of high glucose. The resulting ROS contribute to damage to podocytes, causing nephropathy.[1,16-18] Considering the above, it is obvious that pharmacological correction of OS in the kidneys in DM may have a beneficial effect on the course of DN. However, the question remains unresolved: Suppressing which of the above mechanisms of intrarenal OS can be most effective in treating DN? To understand this issue, in the first stage of our study we decided to evaluate the effectiveness of correcting the direct prooxidant effect of hyperglucosuria, as one of the first mechanisms in the formation of OS in DN. For this, we chose the classical direct antioxidant ATA as a pharmacological tool.

The aim of this study was to evaluate the effect of ATA on the activity of FRO in renal tissue and renal function in rats with experimental streptozotocin (STZ)-induced DM.

## Materials and Methods

Experiments were conducted on 22 male Wistar rats aged 60-100 days and weighing 250-300g. The work with animals was carried out in accordance with the principles of humanism laid down in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, in accordance with the "Animal experimentation legislations."

The animals were divided into two groups. In Group 1 (control) (n=10), to induce DM, the animals were injected intraperitoneally 1ml of STZ solution in the citrate buffer at a dose of 65mg/kg. For more selective modeling of type 2 DM, the rats were previously injected with an intraperitoneal solution of cytoflavin based on a nicotinamide dose of 115 mg/kg..[19] In Group 2 (experimental) (n=12), DM was simulated in a similar way and ATA was administered. In preliminary studies, we have shown that typical signs of nephropathy in rats, including OS in the kidneys, develop as early as 4 weeks after the administration of STZ.[20] At the same time, we also found that in rats with 8-month DM, pathological changes in the kidneys become extremely pronounced and, most likely, irreversible.[21] The results obtained show that the most reliable assessment of the effectiveness of pharmacological correction of DN is possible mainly in the early stages of DN, and therefore the total duration of the experiment in this study was 8 weeks (4 weeks of *STZ*-induced DM, then another 4 weeks - treatment). In Croup 2, ATA was administered in the period from the fifth to eighth weeks, inclusive, intragastrically through a tube at a daily dose of 300 mg/kg. This dose was chosen based on the results of our previous experiments to study the effect of ATA on the course of experimental oxalate nephrolithiasis.[22] In both groups, before starting diabetes modeling, and then weekly, we determined the concentration of glucose, protein, and creatinine in the urine, and their urinary excretion was calculated.

In urine, the concentration of glucose, protein, and creatinine was determined on the automatic biochemical analyzer DIRUICS-T240 using commercial biochemical kits (DIAKON-DS, Russia). After 8 weeks of the experiment, the rats were euthanized under ethereal anesthesia and both kidneys were extracted, one of which was used for morphological research, and the other one was used to determine the biochemical markers of oxidative stress.

The activity of FRO in the kidneys of the rats was assessed by measuring the concentration of TBRP in the renal tissue homogenate and the activity of antioxidant enzymes (SOD, CAT, GPx).[15,22] The morphological study was carried out according to the scheme we approved in previous experiments.[20]

Statistical analysis was performed using using a special program Statistica 13.3.1 (license JPZ906I448517FAACD-K). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean (standard error of the mean [SEM]); non-normal variables were reported as median (Me) and interquartile range (IQR; 25th to 75th percentiles). The Mann-Whitney U Test was used to compare the differences between the two independent groups. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of $P<0.05$ was considered statistically significant.

## Results

Experiments showed that after a 4-week course of ATA, the TBRP concentration in the kidney tissues of the rats in Group 2 was 5.3 times lower than in Group 1. The activity of all antioxidant enzymes did not differ between the two groups (Table 1).

***Table 1.***
***Indicators of activity of FRO in the kidneys of the rats***

| Variable | Group 1 (Control) | Group 2 (Experiment) | *P*-value |
|---|---|---|---|
| TBRP concentration (μmol/mg) | 10.1 (8.3;13.5) | 1.9 (1.6;2.3) | <0.0000 |
| CAT activity (%) | 9.8 (6.2;27.1) | 9.5 (7.7;24.1) | >0.05 |
| SOD activity (%) | 6.8 (4.7;8.5) | 6.5 (4.8;10.3) | >0.05 |
| GPx activity (%) | 34.7 (31.4;37.3) | 37.2 (34.0;40.0) | >0.05 |

In Group 1, during all 8 weeks of the experiment, the levels of renal excretion of glucose, protein, and creatinine significantly exceeded the initial level, while the level of diuresis remained stable (Table 2). In Group 2, despite the course of treatment, the described parameters did not differ from Group 1.

The results of a morphological study showed that after 8 months of *STZ*-induced DM, the kidney glomeruli of the Group 1 animals were enlarged. In addition, there was a significant expansion of the intercapillary space due to the accumulation of Schiff-positive mesangium and connective tissue.

***Table 2.***

***Indicators of excretory kidney function in the two groups***

| | Diuresis (ml/day) | | Excretion of glucose (μmol / day) | | Protein excretion (mg / day) | | Excretion of creatinine (μmol / day) | |
|---|---|---|---|---|---|---|---|---|
| | Group 1 (Control) | Group 2 (Experiment) | Group 1 (Control) | Group 2 (Experiment) | Group 1 (Control) | Group 2 (Experiment) | Group 1 (Control) | Group 2 (Experiment) |
| Initial level | 1.6 (1.0;2.0) | 1.2 (1.0;1.8) | 0.1 (0.02;0.38) | 0.1 (0.02;0.53) | 7.3 (1.7;7.9) | 6.0 (4.2;9.5) | 18.3 (1.9;26.1) | 18.1 (12.822.7) |
| Week 1 | 1.8 (1.4;2.2) | 2.0 (1.5;2.8) | 10.8 (28.6;12.2) *P*=0.003 | 10.0 (5.3;15.5) *P*=0.002 | 10.9 (8.7;24.4) *P*=0.016 | 13.5(9.9;15.3) *P*=0.004 | 32.9 (28.5;41.0) *P*=0.016 | 37.1 (26.2;48.6) *P*=0.008 |
| Week 2 | 1.6 (1.2;2.4) | 2.0 (1.1;2.4) | 0.8 (0.4;3.6) *P*=0.026 | 1.7(0.8;3.6) *P*=0.003 | 7.7 (6.1;14.1) | 10.5 (7.6;12.4) *P*=0.012 | 25.2 (23.6;34.8) *P*=0.033 | 35.1 (21.4;39.2) *P*=0.006 |
| Week 3 | 1.6 (1.4;2.0) | 1.7 (1.1;1.9) | 1.3 (0.8;1.9) *P*=0.009 | 1.5 (1.2;2.2) *P*=0.013 | 10.8 (7.3;13.5) *P*=0.013 | 9.3 (7.8;11.5) *P*=0.034 | 46.6 (24.1;51.3) *P*=0.013 | 42.7 (31.6;44.7) *P*=0.003 |
| Week 4 | 1.9 (1.6;2.2) | 1.9 (1.3;2.1) | 1.1 (1.0;2.3) *P*=0.028 | 0.8 (0.5;1.6) *P*=0.019 | 10.2 (8.1;15.9) *P*=0.022 | 10.0 (8.5;11.2) *P*=0.015 | 36.3 (29.1;52.3) *P*=0.017 | 35.2 (26.2;45.6) *P*=0.002 |
| Week 5 | 1.8 (1.2;2.8) | 1.7 (1.0;1.9) | 1.7 (1.0;1.9) | 0.6 (0.3;1.6) *P*=0.015 | 10.9 (6.9;12.1) *P*=0.017 | 9.6 (6.1;12.2) | 30.4 (11.5;46.8) *P*=0.047 | 21.1 (10.6;32.6) |
| Week 6 | 1.6 (0.8;2.2) | 1.4 (0.8;2.0) | 2.2 (1.6;4.8) *P*=0.009 | 2.2 (1.5;3.2 *P*=0.002 | 8.4 (4.6;9.7) | 7.9 (6.3;11.9) | 29.5 (18.4;50.5) *P*=0.022 | 30.4 (18.9;39.6) *P*=0.023 |
| Week 7 | 1.2 (1.0;1.4) | 1.0 (0.9;1.5) | 1.2 (0.8;1.3) *P*=0.037 | 1.4 (1.0;2.2) *P*=0.012 | 7.4 (7.1;10.7) *P*=0.047 | 7.1 (5.6;9.6) | 33.1 (24.8;49.9) *P*=0.009 | 34.2 (28.1;48.0) *P*=0.002 |
| Week 8 | 1.3 (0.8;2.0) | 1.0 (0.7;1.5) | 1.5 (0.8;2.2) *P*=0.017 | 1.7 (0.9;2.7) *P*=0.010 | 10.3 (7.4;13.5) *P*=0.017 | 11.6 (5.9;14.5) | 34.2 (21.0;49.7) *P*=0.013 | 35.9 (19.1;45.7) *P*=0.019 |

*P – level of significance with the initial data*

The basal membranes of the glomeruli capillaries were significantly thickened, the capillary lumen was narrowed. The capsule of the kidney glomeruli looked thickened. Glomeruli capillaries were full-blooded. In the kidney interstitium, there were foci of lymphoplasmacytic infiltration. The tubular lumens were expanded; the basal membranes of the tubulars were thickened. Nephrocytes were in a state of hyaline-drop dystrophy. The walls of the arteries were thickened; the elastic membranes were hyperplastic. The blood vessels were in a state of plethora (Fig 1).

In the Group 2 animals, the size of the kidney glomeruli decreased slightly, compared to Group 1; the intercapillary space was expanded due to the accumulation of Schiff-positive mesangium and connective tissue. The basal membranes of the glomeruli capillaries were thickened, the capillary lumen was narrowed. The capsule of the kidney glomeruli looked thickened. Glomeruli capillaries were full-blooded. In the kidney interstitium, there were single foci of lymphoplasmacytic infiltration. The tubular lumens were expanded; the basal membranes of the tubulars were thickened. Nephrocytes were in a state of hyaline-drop dystrophy. The walls of the vessels were moderately thickened; the elastic membranes were hyperplastic (Fig.2).

Table 3 presents the quantitative indicators of the morphometric study of the kidneys of both groups of rats. Statistically significant differences between groups were not identified.

The question of whether oxidative stress is primarily responsible for the diabetic complications was extensively investigated, but remained unanswered. The results obtained, to a certain extent, were unexpected. On the one hand, ATA exerted its direct antioxidant effect in the kidneys, as evidenced by a fivefold decrease (relative to the control group) in the concentration of TBRP, the main marker of membrane phospholipid peroxidation. At the same time, ATA had no effect on the activity of antioxidant enzymes, which is also in good agreement with the direct non-enzymatic nature of the antioxidant effect of ATA.

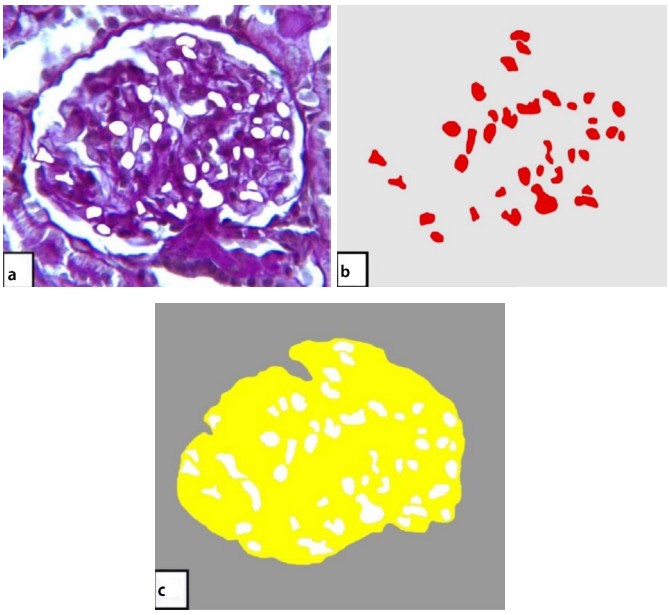


***Fig. 1.*** *Control group.*
*(a) - Kidney glomerulus; (b) - Luminal narrowing of capillaries; (c) - Increased mesangial area. McManus staining method; computer processing of photomicrographs (×1200).*

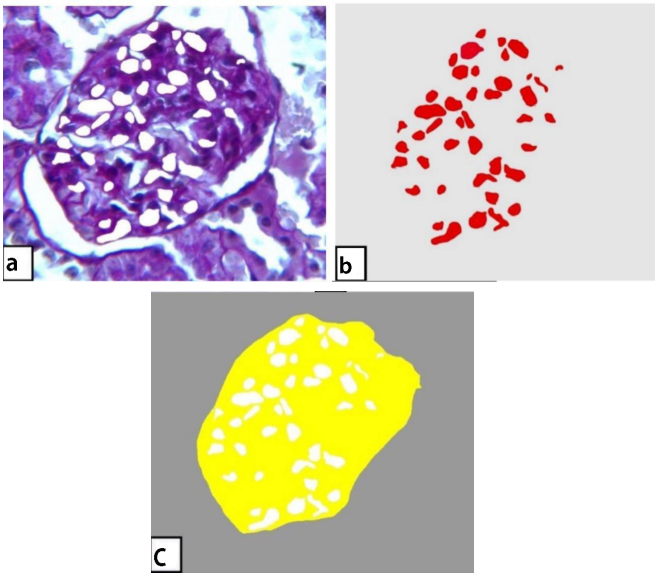


***Fig. 2.*** *Experimental group.*

*(a) - Kidney glomerulus; (b) - Luminal narrowing of capillaries; (c) - Increased mesangial area. McManus staining method; computer processing of photomicrographs (×1200).*

***Table 3.***

***The quantitative indicators of the morphometric study of the kidneys of both groups of rats***

| Indicators | Group 1 (Control) | Group 2 (Experiment) | P-level |
|---|---|---|---|
| The area of the renal glomeruli (μm²) | 7089.5±262.7 | 6316.3±115.5 | >0.05 |
| The total area of blood vessels in the glomerulus (μm²) | 1064.6±115.5 | 953.3±112.5 | >0.05 |
| Glomerular capillary lumen area (μm²) | 22.65±1.8 | 23.25±2.1 | >0.05 |
| Mesangium area in the glomeruli (μm²) | 5396.6±85.8 | 4548.1±115.5 | >0.05 |
| The number of podocytes in the glomerulus | 28.2±2.9 | 34.3±2.3 | >0.05 |

At the same time, ATA did not cause any significant changes in the development of experimental pathology. A high level of proteinuria and glomerular filtration rate persisted, and morphological changes in tissues and cells of the renal glomeruli were identical to the control.

The results obtained suggest that the suppression of the direct component of the oxidative effect of glucose and its metabolic products is insufficient to normalize the structure and function of the renal filtration barrier. It is possible that the mediated mechanisms of oxidation make a much more significant contribution to the development of nephropathy. In this regard, the search for effective methods of pharmacological correction of DN implies further study of the effects of OS on the development of nephropathy.

It should be noted that the effect of ATA on the course of DN has been studied by various researchers. There are a number of studies showing the effectiveness of this drug as an antioxidant in the treatment of DN.[23-25] At the same time, other authors believe that the role of ATA in the correction of DN is controversial and needs to be studied further. The results of our study showed that ATA, while exerting a pronounced suppression of FRO in the kidneys in experimental DM, did not contribute to the correction of DN.

**In conclusion**, the long-term administration of ATA in experimental streptozotocin (STZ)-induced DM is accompanied by a significant suppression of the activity of the FRO processes in the kidneys, but does not lead to an improvement in the course of DN.

## Competing Interests

The authors declare that they have no competing interests.

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