# Effect of Thymoptin on Hemostasis and Fibrinolysis in Rats with Experimental Nephritis

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Immune nephritis in rats was induced by administration of nephrotoxic rabbit antiserum. The development of severe renal inflammation (proteinuria, edema, lipemia, increased erythrocyte sedimentation rate, and 30% mortality) was accompanied by hypercoagulation and inhibition of fibrinolysis. Repeated subcutaneous injections of thymoptin in a low dose of 0.1 μg/200 g (5 injections every other day) increased the severity of inflammation and prethrombotic state of the blood. Lengthening the period between injections (5 injections at 5-day intervals) was followed by a tendency toward attenuation of nephritis and correction of hypercoagulation. In healthy rats, thymoptin produced an opposite effect on hemostasis, which was manifested in moderate stimulation of fibrinolysis and hypocoagulation.

**Key Words:** *thymoptin; immune nephritis; hypercoagulation; hemostasis; fibrinolysis*

Regulatory peptides are the major constituents of various pharmaceutical preparations. However, general physiological properties of peptides (polyfunctionality and pleiotropism) limit the use of these compounds in medical practice. Detailed, large-scale, and long-term preclinical and clinical trials should take into account a variety of functional influences of peptides, their interrelations, dose dependence, route of administration, and long-term or delayed effects. It mainly concerns peptides of the immune system, including thymic peptides. Thymus regulating primarily cell-mediated immunity, is the source of functionally similar active preparations (*e.g.*, thymosin [7], thymalin [2], and thymoptin [1]) with high immunostimulatory activity. Our previous studies showed that thymoptin in low doses (0.1 μg/200 g and 1 μg/200 g) improves the system of hemostasis and fibrinolysis in healthy animals [1]: it slightly decreases hypercoagulation, stimulates fibrinolytic activity, and lyses small experimental thrombi. These properties of thymoptin are of considerable importance for medical practice. We assumed that thymoptin can be used for the treatment of inflammatory immune diseases accompanied by hypercoagulation. One of these diseases is glomerulonephritis. Immune damage to the kidneys in glomerulonephritis is accompanied by inhibition of fibrinolysis and hypercoagulation [3,6].

Here we studied the correcting effect of thymic preparation thymoptin on hemostasis and fibrinolysis in rats with experimental nephritis.

## MATERIALS AND METHODS

Masugi nephrotoxic nephritis in rats (modification of N. V. Nikiforova [4]) was induced by single intravenous injection of rabbit antiserum. The antiserum was obtained by immunization of rabbits with homogenate of rat renal cortex. The development of renal injury and inflammation was estimated from the increase in urine protein concentration (proteinuria) and several clinical signs of the dis-

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ease, including edema, polyuria and oliguria, diarrhea, lipemia, and elevation of erythrocyte sedimentation rate.

Two series were performed on 120 outbred albino rats. In series I ($n$=60), Masugi nephritis was induced in 40 rats. Twenty of these animals (treatment group 1) received no therapy. Twenty rats (group 2) received thymopotin in a dose of 0.1 μg/200 g (5 subcutaneous injections every other day). The control groups consisted of 10 intact rats (control group 1) and 10 healthy animals receiving thymopotin (0.1 μg/200 g, 5 subcutaneous injections every other day). The study was conducted for 45 days. In series II, thymoptin was administered at 5-day intervals. The clinical course of the disease was monitored. Biochemical tests were performed with the blood and urine. Urinary protein concentration was measured by the sulfacyl method. The following parameters of hemostasis and fibrinolysis were determined: fibrinogen concentration; soluble fibrin monomer complexes (SFMC); and antithrombin III (AT-III) activity, plasma euglobulin lysis time (ELT), plasminogen activator (PA) activity, and content of fibrinolysis inhibitor $\alpha_2$-antiplasmin ($\alpha_2$-AP). All methods were described elsewhere [4].

## RESULTS

Symptoms of nephritis were revealed 1 day after administration of nephrotoxic serum. The animals were inert and refused food. They were characterized by limb edema and diarrhea. The development of renal damage and progression of the disease in rats of the treatment group were estimated from an increase in proteinuria. The dynamics of urine protein concentration in animals with nephritis is shown in Figure 1. Urine protein concentration increased by 13.5 times by the 3rd day and reached maximum on days 7-10. This parameter then decreased, but did not return to the baseline level by day 45. Signs of inflammation included increased erythrocyte sedimentation rate and sharp increase in the content of acute phase proteins (fibrinogen and $\alpha_2$-AP, Table 1). Inflammation was accompanied by a sharp increase in hypercoagulation activity of the blood: the concentrations of fibrinogen and SFMC increased, while antithrombotic reserves were exhausted, *e.g.* AT-III concentration decreased 2-fold by day 10 (Table 1). Inhibition of fibrinolysis contributed to progression of the prethrombotic state: ELT and AP activity sharply decreased, while $\alpha_2$-AP concentration increased by 10-12 times (Table 1). Similar changes in hemostasis and fibrinolysis accompanied by nephrotic syndrome were observed in patients with glomerulonephritis [6,9]. The dynamics of hemostasis parameters with progression of the disease and dynamics of proteinuria were similar (Fig. 1, Table 1): maximum on day 10 of the disease and normalization by day 45.

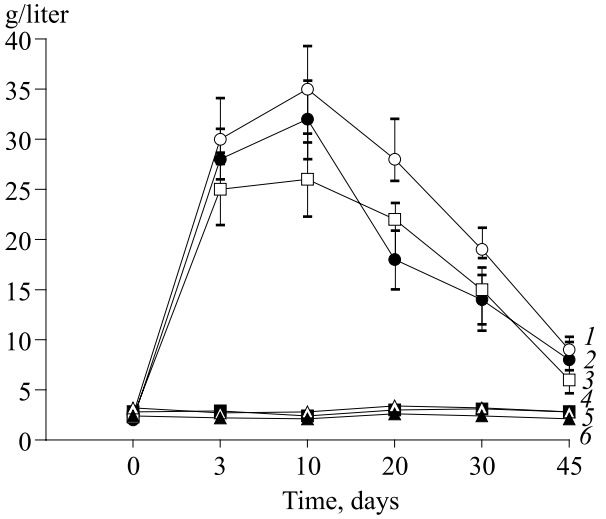


**Fig. 1.** Urine protein concentration in rats with nephrotoxic nephritis. Nephrotoxic nephritis (*1*); nephrotoxic nephritis and 5-fold administration of thymoptin at daily intervals (*2*); nephrotoxic nephritis and 5-fold administration of thymoptin at 5-day intervals (*3*); intact animals (*4*); control animals after 5-fold administration of thymoptin at daily intervals (*5*); control animals after 5-fold administration of thymoptin at 5-day intervals (*6*).

Thymoptin produced a paradoxical effect. Instead of expected improvement of the general state (31% mortality rate) and normalization of hemostasis and fibrinolysis, the preparation administered

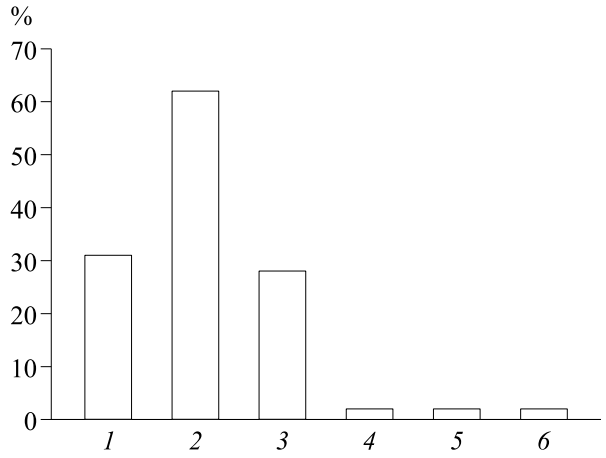


**Fig. 2.** Mortality rate in animals with nephrotoxic nephritis. Nephritis without treatment (*1*); nephritis and 5-fold administration of thymoptin every other day (*2*); nephritis and 5-fold administration of thymoptin at 5-day intervals (*3*); intact animals (*4*); control animals after 5-fold administration of thymoptin at daily intervals (*5*); control animals after 5-fold administration of thymoptin at 5-day intervals (*6*).

**TABLE 1.** Parameters of Hemostasis and Fibrinolysis in Rats with Nephrotoxic Nephritis Receiving 5 Injections of Thymoptin Every Other Day

| Group, day of observation | Hemostasis | | | Fibrinolysis | | |
|---|---|---|---|---|---|---|
| | Fibrinogen, g/liter | SFMC, g/liter | AT-III, % | ELT, min | PA, $mm^2$ | $\alpha_2$-AP, % |
| Nephritis ($n$=20) | | | | | | |
| 0 | 320±35 | 0.050±0.006 | 105.0±11.3 | 210±24 | 32±4.4 | 100 |
| 3 | 1150±120*** | 0.12±0.02** | 68.0±7.4** | 1020±110*** | 16.2±2.8 | 1206±106*** |
| 7 | 1200±150*** | 0.130±0.025** | 52.0±6.3** | 1040±96*** | 6±1** | 1020±110*** |
| 20 | 810±101** | 0.09±0.01* | 59.0±7.5** | 830±94*** | 9±1* | 810±91*** |
| 30 | 610±72* | 0.08±0.01* | 75.0±7.8* | 610±74** | 15.0±1.8 | 310±26* |
| 45 | 501±61* | 0.07±0.01* | 80.0±7.8 | 450±74 | 32.0±4.4 | 162±21 |
| Nephritis+ thymoptin ($n$=20) | | | | | | |
| 0 | 330±35 | 0.060±0.007 | 106.0±12.0 | 210±24 | 14.0±0.4 | 100 |
| 3 | 1180±129*** | 0.130±0.022** | 64.0±6.3** | 1090±110*** | 0.5±0.1** | 1220±126*** |
| 7 | 1300±151*** | 0.150±0.021** | 42.0±6.4** | 1305±140*** | 0.8±0.1** | 1360±127*** |
| 20 | 920±101*** | 0.120±0.015** | 69.0±6.7** | 990±94*** | 6.0±0.6** | 910±105*** |
| 30 | 710±96** | 0.090±0.012* | 71.0±7.8* | 805±81** | 10.0±1.4* | 360±44** |
| 45 | 620±76* | 0.09±0.01* | 72.0±7.4 | 510±54 | 10.0±1.2* | 260±34* |
| Control ($n$=10) | | | | | | |
| 0 | 320±35 | 0.050±0.006 | 105.0±11.3 | 210±24 | 29.0±3.6 | 100 |
| 3 | 390±44 | 0.065±0.007 | 110.0±12.1 | 320±29 | 34.0±4.3 | 98.0±9.9 |
| 7 | 410±45 | 0.071±0.008 | 96.0±10.5 | 290±31 | 26.0±3.3 | 102±10 |
| 20 | 380±42 | 0.062±0.080 | 84.0±9.5 | 310±34 | 30.0±4.1 | 101±11 |
| 30 | 460±53 | 0.050±0.006 | 91.0±9.4 | 280±30 | 22.0±3.5 | 110±11 |
| 45 | 410±54 | 0.059±0.007 | 96.0±8.9 | 320±29 | 21.0±2.9 | 105±12 |
| Control+ thymoptin ($n$=10) | | | | | | |
| 0 | 330±35 | 0.065±0.006 | 105.0±11.3 | 213.0±21.4 | 33.0±4.1 | 100 |
| 3 | 330±36 | 0.050±0.007 | 99.6±11.2 | 220±25 | 30.0±3.9 | 94±11 |
| 7 | 270±29* | 0.042±0.005 | 105.0±10.6 | 210±23 | 46.0±3.8* | 96±10 |
| 20 | 230±31* | 0.034±0.004* | 98.0±9.8 | 170±21* | 38.0±3.6* | 84±9* |
| 30 | 240±31* | 0.041±0.005 | 96.0±9.3 | 205±28 | 29.0±3.4 | 91±10 |
| 45 | 310±33 | 0.046±0.006 | 110.0±11.2 | 180±21 | 28.0±3.5 | 94±11 |

**Note.** Here and in Table 2: *$p$<0.05, **$p$<0.01, and ***$p$<0.001 compared to the control.

according to accelerated scheme (5 injections every other day) aggravated the course of nephritis and increased mortality rate (by 2 times, Fig. 2), proteinuria, lipemia, and erythrocyte sedimentation rate. Aggravation of nephritis was accompanied by an increase in hypercoagulation and inhibition of fibrinolysis (Table 1). These changes became most pronounced on day 10 and persisted for 45 days.

The effect of thymoptin in healthy rats was opposite to that in rats with nephritis. These data are consistent with the results of previous studies [1]. The decrease in fibrinogen concentration, stable level of SFMC, and slight activation of fibrinolysis (Tables 1 and 2) indicate that thymoptin in low doses has a moderate stimulatory effect on antithrombotic and fibrinolytic properties of the blood in healthy control animals.

Hence, immunostimulatory compound thymoptin in a low dose produced opposite effect on healthy animals and animals with nephritis.

Administration of thymoptin in series II had a more favorable effect on rats. The severity of immune inflammation in the kidneys (proteinuria and

**TABLE 2.** Parameters of Hemostasis and Fibrinolysis in Rats with Nephrotoxic Nephritis after Fivefold Administration of Thymoptin at 5-Day Intervals

| Group, day of observation | Hemostasis | | | Fibrinolysis | | |
|---|---|---|---|---|---|---|
| | Fibrinogen, g/liter | SFMC, g/liter | AT-III, % | ELT, min | PA, $mm^2$ | $\alpha_2$-AP, % |
| Nephritis ($n$=20) | | | | | | |
| 0 | 320±35 | 0.050±0.006 | 105.0±11.3 | 210±24 | 32.0±4.4 | 100 |
| 3 | 1150±120*** | 0.12±0.02** | 68.0±7.4** | 1020±110*** | 16.2±2.8 | 1206±106*** |
| 7 | 1200±150*** | 0.130±0.025** | 52.0±6.3** | 1040±96*** | 6±1** | 1020±110*** |
| 20 | 810±101** | 0.09±0.01* | 59.0±7.5** | 830±94*** | 9±1* | 810±91*** |
| 30 | 610±72* | 0.08±0.01* | 75.0±7.8* | 610±74** | 15.0±1.8 | 310±26* |
| 45 | 501±61* | 0.07±0.01* | 80.0±8.2 | 450±42 | 32.0±4.4 | 162±21 |
| Nephritis+ thymoptin ($n$=20) | | | | | | |
| 0 | 320±35 | 0.050±0.006 | 105.0±11.5 | 210±24 | 36.0±4.6 | 100 |
| 3 | 1120±110*** | 0.12±0.02** | 72.0±8.4* | 1010±105*** | 16.0±2.4** | 1220±120*** |
| 7 | 1220±123*** | 0.100±0.022** | 65.0±7.6** | 920±101*** | 14.0±2.2* | 1020±106*** |
| 20 | 701±69** | 0.08±0.01* | 69.0±7.5* | 710±72** | 10.0±1.4** | 620±74** |
| 30 | 580±71* | 0.050±0.006* | 75.0±7.8* | 510±60* | 12.0±1.6* | 305±33* |
| 45 | 410±45 | 0.060±0.007 | 89±9 | 410±39 | 16.0±1.8 | 145±18 |
| Control ($n$=10) | | | | | | |
| 0 | 320±35 | 0.050±0.006 | 105.0±11.3 | 210±24 | 29.0±3.6 | 100 |
| 3 | 390±44 | 0.065±0.007 | 110.0±12.1 | 320±29 | 34.0±4.3 | 98.0±9.9 |
| 7 | 410±45 | 0.071±0.008 | 96.0±10.5 | 290±31 | 26.0±3.3 | 102±10 |
| 20 | 380±42 | 0.062±0.080 | 84.0±9.5 | 310±34 | 30.0±4.1 | 101±11 |
| 30 | 460±53 | 0.050±0.006 | 91.0±9.4 | 280±30 | 22.0±3.5 | 110±11 |
| 45 | 410±54 | 0.059±0.007 | 96.0±8.9 | 320±29 | 21.0±2.9 | 105±12 |
| Control+ thymoptin ($n$=10) | | | | | | |
| 0 | 305±41 | 0.043±0.005 | 101.0±10.5 | 220±29 | 36.0±4.8 | 100 |
| 3 | 290±32 | 0.048±0.005 | 95.0±9.4 | 195±25 | 38.0±4.6 | 105±12 |
| 7 | 280±29 | 0.042±0.005 | 110.0±10.6 | 190±23 | 49.0±4.4* | 98±12 |
| 20 | 210±24* | 0.031±0.004* | 112.0±10.7 | 165±21* | 43.0±3.6 | 92±10 |
| 30 | 260±29 | 0.035±0.003 | 106.0±9.4 | 150±21* | 29.0±3.4 | 98±10 |
| 45 | 280±31 | 0.049±0.005 | 110.0±11.2 | 180±23* | 28.0±4.1 | 91±11 |

death of animals), activation of hemostasis, and inhibition of fibrinolysis were less pronounced under these conditions compared to not only animals treated with thymoptin in series I, but also control animals with nephritis (Figs. 1 and 2, Table 2). These changes were statistically insignificant, but the tendency to the improvement and correction of the test parameters indicates that thymoptin can be used for creation of a drug for the treatment of prethrombotic state.

Thymoptin produced a toxic effect in series I, which is probably related to its role in the pathogenesis of immune nephritis. The damaging effect of the antigen-antibody complex is the major pathogenetic factor of this disorder. Our preparation probably stimulates antibody production, which is consistent with published data [8]. Administration of thymoptin with long intervals provides the specific effect of this compound. Thymoptin stimulates T lymphocytes to synthesize, accumulate, and release plasminogen activator, plasminogen, and other profibrinolytic proteins [10].

It should be emphasized that thymoptin has the opposite effect on treated and healthy animals. Moreover, the action of thymoptin on treated animals depended on the scheme of administration of

this compound. Hence, the therapeutic effect of thymoptin can be modified by varying the dose and scheme of treatment.

Our results indicate that experimental immune nephritis in rats due to single intravenous injection of nephrotoxic serum is accompanied by hypercoagulation and inhibition of fibrinolysis (days 3-45 after treatment). The thymic preparation thymoptin in a dose of 0.1 μg/200 g moderately stimulates fibrinolysis and hypercoagulation in healthy animals. Administration of thymoptin every other day increased the severity of inflammation and hypercoagulation in animals with experimental nephritis. The toxic effect was less significant after 5-fold treatment with thymoptin at 5-day intervals (as compared to series I). Under these conditions, thymoptin improved the state of animals and parameters of hemostasis and fibrinolysis.

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