EFFECT OF TIMOSIN AND CATTLE LIVER HYDROLYSATE ON THE NUMBER OF ANTIBODY-FORMING CELLS IN THE SPLEEN OF MICE WITH SECONDARY IMMUNODEFICIENCY INDUCED BY CCL_4 TOXIC HEPATITIS

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Annotation. This study investigated the effects of Timosin and cattle liver hydrolysate on the number of antibody-forming cells (AFCs) in the spleen of mice with secondary immunodeficiency induced by carbon tetrachloride (CCl_4)-induced toxic hepatitis. Experiments were conducted on outbred white mice. Toxic hepatitis was induced by intraperitoneal administration of CCl_4 dissolved in olive oil. Immunological parameters were assessed using the plaque-forming cell assay based on the local hemolysis reaction. The results demonstrated that CCl_4 administration caused an 8.9-fold decrease in the number of antibody-forming cells compared with the healthy control group. Treatment with Timosin and cattle liver hydrolysate promoted the restoration of humoral immune responses. The most pronounced immunostimulatory effect was observed with a 1% solution of cattle liver hydrolysate. These findings suggest that cattle liver hydrolysate may be considered a promising biostimulatory agent for correcting secondary immunodeficiency associated with toxic hepatitis.

Keywords: toxic hepatitis, carbon tetrachloride (CCl_4), secondary immunodeficiency, Timosin, cattle liver hydrolysate, immunomodulator, biostimulator, antibody-forming cells, spleen, immunity.

Introduction. The liver plays a crucial role in the regulation of metabolic and immunological processes in the body. Various toxic agents, particularly carbon tetrachloride (CCl_4), can induce toxic hepatitis, resulting not only in hepatocellular damage but also in suppression of immune system function [4]. Such conditions are characterized by the development of secondary immunodeficiency, leading to a significant reduction in immune responsiveness [1].

Currently, the study of immunomodulatory and biostimulatory agents capable of restoring immune function under pathological conditions remains an important area of biomedical research. In this regard, evaluating the effects of Timosin and cattle liver hydrolysate on antibody production during secondary immunodeficiency caused by CCl_4-induced toxic hepatitis is of considerable scientific interest [2].

The aim of this study was to investigate the influence of Timosin and cattle liver hydrolysate on the number of antibody-forming cells in the spleen of mice with secondary immunodeficiency induced by CCl_4 toxic hepatitis [3].

Materials and Methods. The experiments were performed on 2–3-month-old outbred white mice of both sexes maintained under standard vivarium conditions with free access to food and water [5].

Toxic hepatitis was induced by intraperitoneal administration of CCl_4 dissolved in olive oil at a dose of 1 ml/kg body weight. Animals were divided into the following experimental groups:

Table 1

Effect of Timosin and Cattle Liver Hydrolysate (Biostimulatory Preparation) on Antibody-Forming Cells in the Spleen of Mice with Secondary Immunodeficiency Induced under Conditions of CCl_4-Induced Toxic Hepatitis

S/n	Experimental group	Number of nucleated cells ($\times 10^9/l$)		Number of antibody-producing cells ($\times 10^9/l$)	
		Total number	Relative change	Total number	Relative change
1.	Healthy control group	185±32		5143	

±872

2.

CCl_4-treated group

169±45*

–1,1

578±103**

–8,9

3.

CCl_4 + Timosin (1 mg/kg

174±28

1,0

1851±300**

+3,2

4.

CCl_4 + cattle liver hydrolysate (0.2%)

172

±26*

+1,1

2623±130**

+4,7

5.

CCl_4 + cattle liver hydrolysate (0.5%)

175±29

+1,2

2677±150**

+1,6

6.

CCl_4 + cattle liver hydrolysate (0.7%)

178±38**

+1,1

2768±128**

+1,2

7.

CCl_4 + cattle liver hydrolysate (0.7%)

180±22**

–1,1

2995±140**

+1,4

Note: *In the control group, mice received an intraperitoneal injection of physiological saline solution (0.9% NaCl) at an equivalent volume. * indicates statistically significant differences in the number of nucleated cells compared with the healthy control group, as well as in the number of antibody-forming cells compared with the group with secondary immunodeficiency induced by CCl_4 at $p < 0.05$. ** indicates $p < 0.01$ ($n = 3–4$).*

To induce the immune response, mice were immunized intraperitoneally with sheep red blood cells (SRBCs) at a dose of 2×10^8 cells per animal. On the fifth day after immunization, the animals were euthanized by decapitation, and their spleens were collected.

Spleen cell suspensions were prepared by homogenization in Hanks' balanced salt solution. The number of antibody-forming cells was determined using a modified Jerne plaque-forming cell assay. Lymphoid cell suspensions were mixed with 10% sheep erythrocytes and complement diluted 1:5, placed into specialized chambers, and incubated at 37°C for 1 hour. The resulting hemolytic plaques were counted

under magnification. Results were expressed per spleen and per 10^6 nucleated cells.

Data were presented as mean ± standard error (M ± m). Statistical significance was accepted at $p < 0.05$ and $p < 0.01$.

Results. The results demonstrated that CCl_4-induced toxic hepatitis markedly suppressed immune function.

In the healthy control group, the total number of nucleated spleen cells was 185 ± 32 × 10^9/L. In mice treated with CCl_4, this value decreased to 169 ± 45 × 10^9/L, representing a 1.09-fold reduction compared with controls.

The number of antibody-forming cells in the control group was 5143 ± 872 × 10^9/L, whereas in the CCl_4-treated group it decreased dramatically to 578 ± 103 × 10^9/L. Thus, CCl_4 administration resulted in an 8.9-fold reduction in antibody-forming cells.

Treatment with Timosin increased the number of antibody-forming cells to 1851 ± 300 × 10^9/L, which was 3.2 times higher than in the untreated CCl_4 group. The number of nucleated cells in this group reached 174 ± 28 × 10^9/L.

Administration of cattle liver hydrolysate also improved immunological parameters. The numbers of antibody-forming cells in the groups treated with 0.2%, 0.5%, 0.7%, and 1.0% hydrolysate solutions were 2623 ± 130, 2677 ± 150, 2768 ± 128, and 2995 ± 140 × 10^9/L, respectively. The highest response was observed in the group receiving the 1% hydrolysate solution.

Similarly, the number of nucleated spleen cells approached normal values, reaching 172 ± 26, 175 ± 29, 178 ± 38, and 180 ± 22 × 10^9/L in the respective treatment groups.

Discussion. The findings indicate that the experimental model of toxic hepatitis induced by CCl_4 causes pronounced immunosuppression. The nearly nine-fold reduction in antibody-forming cells reflects a substantial impairment of humoral immunity.

Administration of Timosin significantly increased the number of antibody-forming cells, confirming its immunomodulatory properties. However, its efficacy was lower than that observed with higher concentrations of cattle liver hydrolysate.

All tested concentrations of cattle liver hydrolysate improved immune parameters. A dose-dependent increase in antibody-forming cell numbers was observed, with the strongest effect achieved using the 1% solution. These results indicate that cattle liver hydrolysate possesses immunoreparative and immunostimulatory activities.

Overall, the findings suggest that cattle liver hydrolysate contributes to the restoration of immune function under conditions of secondary immunodeficiency associated with toxic hepatitis.

Conclusion:

1. CCl_4-induced toxic hepatitis caused secondary immunodeficiency in mice, resulting in an 8.9-fold reduction in the number of antibody-forming cells in the spleen.
2. Timosin increased the number of antibody-forming cells by 3.2-fold compared with the untreated CCl_4 group, demonstrating significant immunomodulatory activity.
3. Cattle liver hydrolysate improved immune parameters at all tested concentrations.
4. The highest efficacy was observed with the 1% hydrolysate solution, which increased the number of antibody-forming cells to $2995 \pm 140 \times 10^9$/L.
5. Cattle liver hydrolysate may be considered a promising biostimulatory agent for correcting secondary immunodeficiency associated with toxic hepatitis.

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