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PHYSIOLOGY

Trekrezan as a Modulator of Hemato- and Immunopoieses

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The list of generally recognized immunoactive preparations do not include synthetic drugs based on the derivatives of arylheteroalkane carbonic acids. However, these compounds, exhibiting a unique biological activity, are promising for creating immunomodulators of a new generation. For example, tris(2-hydrozyethyl)ammonium 2-methyl-phenoxyacetate (trekrezan) is known as an adaptogen with a broad spectrum of activities [1]. This preparation and its close structural analogues display pronounced cancerostatic, protecting, and adaptive activities [2]. At the same time, it was shown that trekrezan is able to stimulate the immunoglobulin M (IgM) response in intact mice with low and high responsiveness to thymus-dependent antigen [3].

We were the first to study the effect of trekrezan on hemato- and immunopoieses in the experimental models of immunopathology. A promising approach to immunocorrection includes the effect of this preparation on the proliferation, differentiation, and self-sustaining of early hematopoietic precursors. The elements of the immune and hematopoietic systems, which have the same precursor (hematopoietic stem cell) and strongly interact with each other, modulate both immuno- and hematopoieses [4].

We used the experimental models based on the graft-versus-host reaction (GVHR) against the background of certain genetic differences between the donor and recipient in allogenic (C57BL/6 → BALB/c → BALB/c) and semiallogenic (DBA/2 → B6D2F1) parent → F_1 systems. The GVHR-induced models of immunity disorders reflect various combined disorders of erythro- and immunopoieses (immunodeficiency combined with hemolytic anemia, immunodeficiency combined with hemolytic anemia and immune complex glomerulonephritis, and immunodeficiency combined with hypoplastic anemia) [5].

It was reported earlier that erythropoietic disorders are involved in the development of the immune-system pathologies in different experimental models: NZB mice (autoimmune disorders), AKR mice (lymphoproliferative diseases), and mice at the age of 24–28 months (senile immunodeficiency) [6, 7]. The restoration of the proliferative activity of early hematopoietic precursors using the inhibitors of hematopoiesis normalizes the immune status in autoimmune, immunodeficiency, and other disorders [8].

In our study, the GVHR-induced model of immunodeficiency and anemia in female (C57BL/6 × DBA/2)F1 (B6D2F1) mice was induced by two intravenous injections (a week apart) of 50×10^6 lymphoid cells obtained from female mice of the parental strain DBA/2. The number of antibody (IgM)-forming cells (AFCs) in the mouse spleen *in vivo* was evaluated on day 4 after the intravenous immunization with 2×10^8 sheep erythrocytes by the number of local hemolysis areas. Hematocrit was determined spectrophotometrically at λ = 405 nm. The hemoglobin content in mouse blood was calculated using a calibration curve. The number of reticulocytes was determined in a blood smear stained with azure II; that of erythrokaryocytes, in a bone-marrow smear stained with azure II–eosin. The number of erythroid burst-forming units (BFU-Es) in the bone-marrow culture was estimated using the standard procedure [9]. The number of BFU-Es was calculated for 10^5 bone-marrow cells (BMCs). The protein content of urine was determined calorimetrically with Kumsai Brilliant Blue dye on a Titertec Multiskan devise at λ = 570 nm. In the experiments, we used mice with persistent proteinuria (protein content in urine was determined more than once).

B6D2F1 mice with GVHR-induced immunodeficiency were characterized by erythropoiesis disorders expressed as peripheral-blood anemia (decreased hemoglobin content and hematocrit) and an increase in the number of early and late erythropoietic precursors (BFU-Es → erythrokaryocytes → reticulocytes). Hemolytic anemia in B6D2F1 mice with immunosuppression was accompanied by the erythron hyperplasia followed by an increase in the number of the hematopoietic precursors BFU-Es, CFU-S-5, and CFU-S-8. For this reason, we studied the effect of a course of trekrezan injections (50 mg/kg, six times a day) on the expression of anemia, the number of early erythropoietic precursors, and the primary humoral immune response (IgM-response) (table). The data summarized in the table indicate that a course of trekrezan injections results in the correction of immunosuppression (a sig-

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Effect of trekrezan on erythro- and immunopoieses in B6D2F1 mice with immunodeficiency and anemia

Parameter	Control	Immunodeficiency	Immunodeficiency + trekrezan
The number of AFCs per spleen	45600	12640*	30826#
Hemoglobin, g/l	189.0	165.6*	191.6#
Hematocrit, %	49.4	45.9*	47.1#
Reticulocytes, %	10.3	15.9*	10.0#
Erythrokaryocytes, %	28.5	34.4*	27.8#
BFU-Es/10^5 BMCs	6.7	20.2*	12.7#

* Significantly different from intact mice; # significantly different from mice with immunodeficiency (according to nonparametric tests).

nificant increase in the number of AFCs) in the spleen and anemia (a significant increase in the hemoglobin content and hematocrit in the blood). As shown in the table, late erythroid precursors in blood and early nucleus-containing erythroid precursors in bone marrow were brought to normal state (the number of reticulocytes and erythrokaryocytes, respectively, significantly decreased).

The data presented in the table also indicate that the trekrezan injections suppressed burst formation in the bone-marrow culture of these mice *in vitro* (the number of BFU-Es/10^5 BMCs significantly decreased). Thus, trekrezan eliminates anemia, thereby decreasing the erythron hyperplasia in the mice with immunosuppression, beginning from early bone-marrow precursors (BFU-Es). This effect of trekrezan on erythropoiesis may result from its direct effect on the erythron cell elements and an indirect (cytokine-mediated) effect. In view of this, we estimated the trekrezan effect on the interleukin-1 (IL1) secretion by the peritoneal macrophages of B6D2F1 mice with GVHR-induced immunodeficiency. We found that trekrezan decreased both the spontaneous (by 38%) and LPS-induced (by 54%) IL-1 production in ill mice. We also showed that spontaneous IL-1 production was enhanced in B6D2F1 mice with immunodeficiency. According to the data reported in [10, 11], IL-1 stimulates the BFU-E growth and the Th2 activity, thus promoting an increase in the antierythrocytic autoantibody production and development of hemolytic anemia. This is indicative of the involvement of the trekrezan-induced decrease in the IL-1 secretion in the elimination of anemia in the mice with GVHR-induced immunodeficiency. In addition, the injections of various doses of trekrezan (single and repeated three times) to intact mouse donors of hematopoietic stem cells (HSCs) significantly suppressed the proliferative activity of HSCs from the bone marrow and spleen, which form colonies on day 8. Addition of trekrezan to the bone-marrow culture of intact mice *in vitro* inhibited burst formation (decreased the number of BFU-Es), i.e., exhibited a direct effect on early erythropoietic bone-marrow precursors.

It is known that IL-1, an anti-inflammatory cytokine, plays a key role in the development of immune inflammation in the kidneys [12]. Taking into account that the IL-1 secretion in the B6D2F1 mice with immunodeficiency, anemia, and immune complex glomerulonephritis is increased, the trekrezan-induced decrease in the IL-1 secretion should decrease the lesion of kidneys.

B6D2F1 mice with immune complex glomerulonephritis received a course of trekrezan injections (5 mg/kg, every other day for 40 days; 15 injections in total). The dynamics of the level of proteinuria was monitored in the same mice weakly. Before trekrezan treatment, proteinuria was detected in 100% of mice (an average protein content in urine was 9.8 mg/ml). Immediately after the termination of trekrezan injections, the protein content in 90% of mice decreased to approximately 5.9 mg/ml. Two months after the termination of the trekrezan injections, the protein content in urine decreased (compared to the initial level) and equaled 4.7 mg/ml, which is significantly lower than the level observed before the treatment.

The GVHR-induced immunodeficiency in male BALB/c mice was induced by a series of transplantations of syngeneic spleen cells immune to alloantigens [13]. In the experiments, we used the mice on month 5–6 of the disease. The moment of GVHR induction (i.e., the last transplantation to the secondary recipients) was taken as the beginning of the disease. Intact male BALB/c mice of the same age served as a control. A course of trekrezan injections (5 mg/kg, every other day for one month, 12 injections in total) to BALB/c mice with GVHR-induced immunodeficiency increased the number of nucleus-containing cells (NCCs) in blood immediately after the end of injections. In the control mice, the number of NCCs in blood was 9.2×10^3 cells/ml; in the mice that received the trekrezan injections, 17.42×10^3 cell/ml. Four months after the termination of trekrezan injections, the number of NCCs in blood of the control mice decreased to 6.9×10^3 cell/ml, whereas in the trekrezan-treated mice, it remained high (13.6×10^3 cell/ml). This is indicative of a persistent stimulatory effect of trekrezan on leukopoiesis.

The development of immune disturbances (immunodeficiencies and autoimmune disorders) is accompanied by shifts not only in the immune system, but also in the erythropoietic system, related to the changes in

the prloliferation of hematopoietic stem cells and the colony-forming activity of hematopoietic precursors of the erythroid and granulocyte–macrophage cells. The data obtained in the study indicate that trekrezan exhibits combined hemato- and immunopoiesis-modulating properties, which opens a new class of immunomodulators that are able to regulate the immune disorders.

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