MECHANISM OF ACTION OF PROPAZINE, A COMPOUND OF THE SYMMETRICAL TRIAZINE GROUP, IN VIVO

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Propazine, a folic acid antagonist, induces macrocytic hypochromic anemia in rabbits, with participation of an autoimmune component in its mechanism, and it disturbs nuclei acid and protein (globin) metabolism. This toxic action of propazine is abolished if exogenous thymidine is administered simultaneously.

Compounds belonging to the symmetrical triazine group are widely used in the treatment of malignancy [1], as immunodepressants during organ and tissue transplantation [4], and also as intermediate products in the chemical industry [7] and in agriculture as pesticides [2].

Derivatives of the symmetrical triazines can act as folic acid antagonists [19]. The ability of such antagonists to induce "thymine starvation" has been described [12]. A typical feature of folic acid deficiency in the body is a disturbance of nucleic acid metabolism [18]. Folic acid antagonists are classed as exogenous etiological factors in the development of hypoplastic and aplastic anemias [3]. Toxicoallergic (immunoaggressive) blood diseases are included by the authors under the same heading. Folic acid avitaminosis is accompanied by glossitis, stomatitis, ulcerative gastritis, and enterocolitis [14].

The object of the present investigation was to study the action of propazine [2-chloro-4, 6-bis-(isopropylamino)symm-triazine] in experiments on rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 29 chinchilla rabbits of both sexes, not less than 2 kg in weight. Daily for a period not exceeding 4 months 15 animals received chemically pure propazine ($C_9H_{16}N_5Cl$, mol.wt. 229.71, readily soluble in vegetable oil and gastric juice, sparingly soluble in water) by mouth as a 25% solution in starch in a dose of 500 mg/kg (0.1 LD_{50}). Four rabbits received the same poison, in the same dose, together with thymidine (50 mg daily), and another four rabbits received only the same dose of thymidine. The results were compared with the corresponding values obtained with intact animals.

The following methods were used: 1) blood analysis in the ordinary way; 2) analysis of the myelogram (erythroid and myeloid series; Pappenheim's panoptic method of staining bone marrow films); determination of the alkali-resistant (fetal) hemoglobin in thrombin-free hemolysates of washed red blood cells and bone marrow cells in the writers' modification for use with the hemoglobin of rabbit red cells [10]; 4) analysis of the hemoglobin fractions in the same substrates by high-voltage analytical electrophoresis for 30 min in agar gel (veronal-medinal buffer, pH 8.6), followed by quantitative estimation of the fractions by densitometry with the ERY-10 apparatus [9]; 5) determination of the soluble proteins [6] and 6) nucleic acids [13] of the bone marrow; 7) detection of antierythrocytic autoantibodies (agglutinins and conglutinins) [16], and hemolysins [11].

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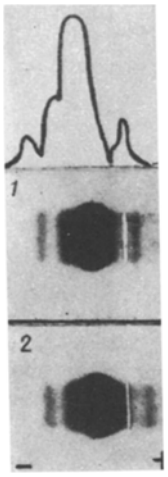

Fig. 1

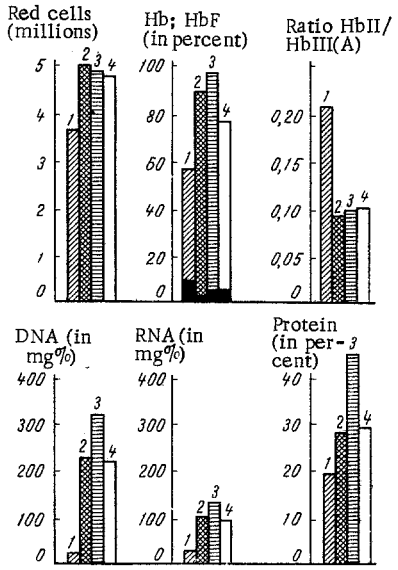

Fig. 2

Fig. 1. Electrophoresis of hemolysates of red cells (1) and bone marrow cells (2) of rabbits with anemia induced by propazine (buffer, pH 8.6).

Fig. 2. Red cells, hemoglobin and its components from the peripheral blood, and soluble proteins, RNA and DNA, from bone marrow of control rabbits and rabbits receiving propazine (1), propazine plus thymidine (2), and thymidine alone (3), and of control animals (4). Part of column shaded black represents HbF.

At the end of the experiments the rabbits were killed by air embolism and autopsied. Pieces of the organs were fixed in 12% neutral formalin, and pieces of the liver in addition in Gendre's mixture. Dewaxed sections were stained with hematoxylin and eosin, sections of the bone marrow (after decalcification of pieces of the sternum) and lymph glands with azure-II-eosin. Liver glycogen was detected with carmine by Best's method and nuclei acids in the brain cells with gallocyanin by Einarson's method. Lipids were detected in frozen sections with Sudan III. Iron in the pigmented deposits of the spleen was demonstrated histochemically by Perles' reaction. The numerical results were subjected to statistical analysis by the Fisher-Student method.

EXPERIMENTAL RESULTS

Administration of propazine was found to induce hypochromic anemia with the appearance of degenerative forms of red cells, macrocytes, and high reticulocytosis, accompanied by a raised ESR and leukopenia on account of an absolute lymphocytopenia and granulocytopenia, with a relative increase in the number of eosinophils and stab cells. Analysis of the myelograms revealed an increase in the number of proerythroblasts, which were giant-sized. Against a background of inhibition of myelopoiesis (a decrease in the number of intermediate forms of myeloid cells and delay in the release of mature granulocytes) giant forms of myelocytes and metamyelocytes were observed. Development of the pathological state was accompanied by increased formation of the fetal type of hemoglobin (HbF). Meanwhile, the level of the principal hemoglobin fraction of rabbits, designated HbIII (A), was reduced, while the content of one of the minor fractions (HbII) was increased. The HbII/HbIII (A) ratio was changed correspondingly. The levels of HbF and of the other fractions in hemolysates of peripheral red blood cells and bone marrow cells were similar (Fig. 1). In the blood serum of ten of the 15 rabbits antierythrocytic antibodies appeared, mainly incomplete heat conglutinins. Antibodies were detected at different times, but not less than 30 days after the original injection of the poison, depending on the degree of development of the anemia, and they continued to be found until the end of the experiments. Besides the disturbances mentioned above, there was a sharp decrease in the level of soluble proteins, DNA, and RNA in the bone marrow of the anemic rabbits (Fig. 2).

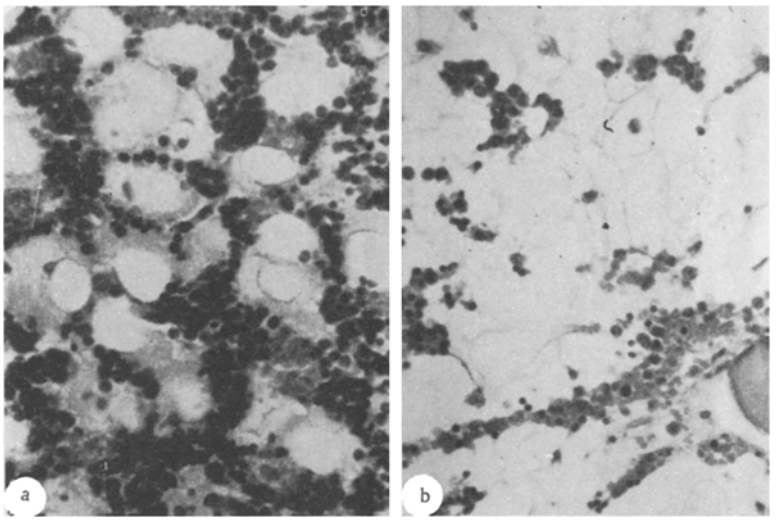

Fig. 3. Effect of propazine on the bone marrow: a) sternal marrow: edema, decrease in total number of hematopoietic cells; b) sternal marrow: sharp decrease in number of hematopoietic cells with disappearance of the parenchyma. Azure-II-eosin, 200 ×.

Autopsy revealed anemia of the internal organs, slight or moderate atrophy of the lymph glands and, as a rule, enlargement of the liver, flabbiness of the ventricle wall, and swelling of the large intestine. The principal microscopic changes were micronecroses, hydropic or fatty degeneration of the liver, glycogen deprivation of the liver, edema of the bone marrow, and a decrease in the number of all hematopoietic cells as the condition progressed, leading ultimately to disappearance of the myeloid tissue (Fig. 3a, and b), extensive focal deposits of hemosiderin in the spleen, edema of the stroma of the lymph glands and mucous membrane of the stomach with areas of necrosis, edema of the mucosa and submucosa of the small and large intestines, and infiltration of the tissues with plasma cells. Histopathological changes consisted of inflammatory changes in the lungs typical of purulent panbronchitis, lobular serofibrinous or purulent pneumonia with abscess-formation, and profuse, mainly perivascular infiltration of the tissue with polymorphs, including eosinophils. Occasional ganglion cells in the nuclei of the cerebellum were deficient in RNA and there were small focal hemorrhages in the white matter of the cerebral hemispheres, myocardium, and kidneys.

In the animals receiving propazine together with thymidine, none of these abnormalities were observed. Administration of thymidine alone led to an increased content of hemoglobin in the circulating blood and an increase in the level of soluble proteins and nucleic acids in the bone marrow (Fig. 2). These findings do not contradict the view that exogenous thymidine participates in DNA synthesis [17], for its endogenous reserves in the cells are small [12].

In the doses used, propazine thus induces the development of various pathological changes in different organs and systems of the rabbit, including hypochromic macrocytic anemia (including a hemolytic component, as shown by the abundant deposits of hemosiderin in the spleen), with a picture resembling that of folic acid deficiency. This last aspect is confirmed by the fact that propazine acts like a folic acid antagonist, preventing a conversion of folic acid into its active form, as direct experiments have shown [5].

Propazine poisoning is accompanied by disturbance of globin synthesis with the production of embryonic forms (HbF) and of HbII, and it also leads to a disturbance of metabolism of amino acids (glycine and serine) playing an important role in globin biosynthesis [8] and to a decrease in the content of soluble proteins and nucleic acids in the bone marrow of the anemic animals. These facts do not contradict the view that hemoglobin synthesis begins at the stage of the polychromatophilic erythroblast and ends at the reticulocyte stage.

A search of the literature revealed only one paper [15] in which changes in hemoglobin synthesis in sexually mature Pekin ducks were found under the influence of a folic acid antagonist (methotrexate). This fact was regarded by the authors as the result of the action of the compound on the gene responsible for

globin synthesis. From the results of the present experiments the writers conclude that this explanation deviates from the fundamental pathological process characterizing the effect of the toxic substances on the body. Blood changes following propazine administration involve an autoimmune component (red cell – antierythrocytic antibodies), and poisoning with this compound leds to the formation of allergic and autoallergic structural changes in the form of infiltration of the lungs with eosinophils in the presence of inflammation.

The toxic action of propazine is abolished by simultaneous administration of exogenous thymidine. This observation could provide the basis for rational methods of prevention and treatment of the effects of poisoning by this compound belonging to the symmetrical triazine group.

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