METHODS

A Comparative Study of Two Models of Hemosiderosis Used for the Investigation of Iron Chelators

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Two mouse models of hemosiderosis are compared. Increased deposition of iron in internal organs is confirmed histochemically. The effectiveness of desferal therapy is evaluated by urinary excretion of iron.

Key Words: *hemosiderosis; histochemistry; desferal*

Problems associated with the treatment of hemosiderosis and hemochromatosis have not been solved. Therefore, the development of effective therapeutic and preventive measures is very important. Desferal, an agent binding and eliminating excessive iron, has been used in clinical practice for more that two decades [4]. However, this preparation has a number of serious disadvantages [2,9,10,11], so the need for safe and effective drugs still remains. A great number of models for the investigation of iron chelators has been developed [3,5,8]. The search for new chelating agents [1] implies the evaluation of effectiveness of potential drugs. We think that the model proposed by C. G. Pitt [7] — induction of hemosiderosis in mice by injection of heterogenic erythrocytes — is one of the most convenient.

Our objective was to compare two mouse models of hemosiderosis: the model developed by Pitt and our model, in which hemosiderosis was initiated by injection of allogenic erythrocytes.

MATERIALS AND METHODS

Experiments were performed on 100 outbred albino mice of both sexes weighing 20-23 g. The animals were divided into equal groups. Group I mice (n=50) were intraperitoneally injected with human packed erythrocytes by the method of Pitt. Group II mice (n=50) were intraperitoneally injected with packed erythrocytes of outbred albino mice. In both groups erythrocytes were injected in doses 0.25, 0.5, and 1.0 ml during a 4-day period. Half of mice in each group were treated with desferal (daily dose 0.5 ml per animal), and the other half mice were injected with normal saline. The mice were decapitated before and at different periods of desferal or normal saline treatment. The effectiveness of treatment was evaluated from the content of iron in the urine and by histochemical methods. The urine was collected during a 4-day period after administration of desferal using replaceable cages. The urinary content of iron was determined by the method of [6] with our modifications. The intensity of hemosiderosis in internal organs was assessed by the histochemical method of Perls after fixation in neutral Formalin and embedding in paraffin.

RESULTS

Administration of human packed erythrocytes to mice in a dose of 0.25 ml per animal resulted in the development of prolonged (1 month) hemosiderosis of internal organs, which was particularly

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TABLE 1. Urinary Excretion of Iron (*M±m*)

Model of hemosiderosis	Therapy	Excreted iron, μg/ mouse/day
Allogenic erythrocytes	Normal saline	0.9±0.6
	Desferal	3.3±1.5
Heterogenic erythrocytes	Normal saline	1.9±0.6
	Desferal	7.3±1.0

intense in the spleen and liver. Siderophages overloaded with blue Perls-positive granules and lumps were present in the liver. In the spleen, intense siderosis of the red pulp was observed. Occasional siderophages were found in the heart, lungs, and kidneys.

Administration of allogenic packed erythrocytes (4 injections) in doses 0.5 and 1.0 ml per animal induced less intense hemosiderosis with deposition of Perls-positive material in Kupffer cells, hepatocytes, red pulp of the spleen, and proximal tubular epithelium of the kidneys. The period of hemosiderosis was much shorter: occasional Perls-positive Kupffer cells were found in the liver two weeks after the last administration of allogenic erythrocytes. It was less pronounced after administration of allogenic erythrocytes in a dose of 0.25 ml.

Table 1 shows urinary excretion of iron after administration of desferal.

Our results show that sufficient degree of hemosiderosis can be achieved in mouse internal organs by administration of allogenic and heterogenic erythrocytes. Hemosiderosis induced by allogenic erythrocytes is less pronounced. There were no differences in the content of Perls-positive material in the internal organs of mice treated with desferal and normal saline. The therapeutic effectiveness of desferal can be evaluated by analysis of urinary excretion of iron. Since our model of hemosiderosis (administration of allogenic erythrocytes) is physiologically more adequate that the model proposed by Pitt and the amount of excreted iron is virtually the same in both models, it can be successfully used for the evaluation of therapeutic effectiveness of new preparations capable of eliminating "pathological" iron from the organism.

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