EFFECT OF PURINE DERIVATIVES ON REGENERATION AND IMMUNOLOGIC REACTIVITY IN ANIMALS

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The effect of azathioprine and KI-7 (a new purine derivative) on immunogenesis and regeneration in mammals was compared. KI-7, which is less toxic than azathioprine, was shown to inhibit antibody synthesis against sheep's red blood cells in mice if injected on the day before or the day of immunization, and also to prolong the life of skin allographs in rabbits considerably compared with the control. It was also shown that during inhibition of the system of immunogenesis, regeneration of the liver still takes place in rats, but the relationship between proliferation and cellular hypertrophy differs from that during regeneration under ordinary conditions and depends on the immunodepressant used. For instance, unlike azathioprine, KI-7 not only did not inhibit mitotic activity of the regenerating liver, but actually stimulated it a little.

KEY WORDS: immunodepressants; allografting; regeneration; mitotic activity.

To inhibit the reaction of transplantation immunity several different chemicals with the property of depressing the function of cells of the lymphoid system are used. However, as experiments by many different workers have shown [3-6], during prolonged administration of chemical immunodepressants severe disturbances arise in the vitally important organs of the recipients as a result of the toxic action of the compound, and this is a serious obstacle to their clinical use. Meanwhile successful transplantation of organs is possible only if immunologic incompatibility between donor and recipients is adequately suppressed and the regenerative processes observed in the graft are sufficiently complete and intensive to ensure healing and long life of the grafts. However, the question of the effect of widely used immunodepressants on regenerative processes still remains almost completely unstudied [1, 2].

Consequently, an important task in allografting of organs and tissues is, first, to seek new and effective chemical agents capable of depressing the response to the transplanted organs without significantly disturbing the viability of the host, and second, the comprehensive studyof the biological action of substances of this type.

This paper describes a comparative study of the effect of azathioprine and of compound KI-7, synthesized at the S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute by P. M. Kochergin, on processes of immunogenesis during skin autografting and on regeneration of the liver. The biological characteristics of KI-7 as an immunodepressant also are described and its dosage is determined.

EXPERIMENTAL METHOD

To study the possibility of the use of KI-7 as an immunodepressant the comparative toxicity of this compound and azathioprine was studied. Toxicity of the substances was determined by Kärber's method on (CBA × C57BL/6)F_1 mice.

The immunodepressive activity of the compounds was studied by two methods: 1) by determining the degree of depression of antibody-forming cells (AFC) in the spleen of CBA mice immunized intraperitoneally with 0.2 ml of a 10% suspension of sheep's red blood cells; the two compounds were injected intraperitoneally in a single dose of 125, 250, 500, and 1000 mg/kg at different times (1 day before, on the same day as, and 1 day after immunization); 2)

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by determining the increase in the survival period of a skin allograft in rabbits receiving KI-7 per os in a dose of 20, 25, or 30 mg/kg and azathioprine in a dose of 25 mg/kg; administration of the compounds began 1-2 days before skin grafting and continued daily for the first 3-4 days after grafting; thereafter they were given at intervals of 1-2 days until the appearance of the first signs of a rejection reaction.

To study the action of KI-7 and azathioprine on regeneration, the liver of sexually mature August rats was studied 22, 48, and 72 h and 7 days after removal of two-thirds of the organ (5 or 6 rats at each time). The experimental animals received a suspension of azathioprine or KI-7 in 1% carboxymethylcellulose (CMC) per os in a dose of 40 mg/kg. The regenerating and intact liver of rats of the same age and body weight, but which received 1% CMC only, served as the control. Thymidine-^{3}H was injected intraperitoneally in a dose of 0.5-1.0 μCi/g 1 h before sacrifice into all the control and experimental animals. The absolute and relative weight of the regenerating liver and the index of labeled nuclei (ILN) were determined. The number of mitotically dividing hepatocytes and the number of binuclear cells were counted. Two diameters of the hepatocytes and their nuclei were measured and their area calculated. The numberical results were subjected to statistical analysis by the Fisher—Student method [1, 2].

EXPERIMENTAL RESULTS

KI-7 was shown to be less toxic than azathioprine: LD_{50} for KI-7 was 700 mg/kg and for azathioprine 340 mg/kg.

Both compounds, if given as a single dose 24 h before, on the day of, or 24 h after immunization with sheep's red cells in doses of 125 and 250 mg/kg, caused very slight inhibition of antibody synthesis compared with the control. A more marked decrease in the number of AFC in the spleens was observed when KI-7 and azathioprine were given in doses of 500 and 1000 mg/kg (Fig. 1). KI-7 and azathioprine, given in a dose of 500 mg/kg the day before immunization, cause an appreciable and statistically significant ($P < 0.01$) inhibition of hemolysin synthesis in the spleen cells; the number of AFC under these circumstances was 682 ± 77.6 and 630 ± 49.5, respectively, compared with 1012 ± 15.9 in the control. When the compounds were given in a dose of 1000 mg/kg a more marked and statistically significant ($P < 0.001$) inhibition of antibody synthesis was observed — to 193 ± 18.4 and 243 ± 23.6 plaques.

After administration of KI-7 and azathioprine to the animals in a dose of 500 mg/kg on the day of immunization the number of AFC was reduced almost by half: to 490 ± 85.7 and 560 ± 49.5, respectively compared with 1012 ± 15.9 in the control. A more marked decrease in the number of AFC in the spleens was observed after administration of KI-7 and azathioprine in a dose of 1000 mg/kg (to 326 ± 47 and 290 ± 42, respectively).

When the compounds were given on the day after immunization with sheep's red cells they had a weak immunodepressive action even when given in a dose of 1000 mg/kg. After treatment of the mice with KI-7 and azathioprine the number of cells synthesizing hemolysin was 710 ± 65.7 and 710 ± 31, respectively.

To sum up the results of these experiments it can be stated that KI-7 and azathioprine are similar in their immunodepressive action on antibody synthesis in immunocompetent mouse spleen cells. The effect of their action increased with an increase in the dose of the compounds. Both immunodepressants, given the day before or on the day of immunization, caused a marked decrease in the number of AFC in the animals' spleens. However, when used in the same dose, but after antigenic stimulation, they had only a weak immunodepressive action.

The study of the immunodepressive action of KI-7 on skin allografts in rabbits showed that KI-7 in a dose of 30 mg/kg considerably prolonged the life of the skin allografts (to 18.1 ± 1.2 days compared with 7.8 ± 0.25 days in the control). In a dose of 25 mg/kg KI-7 had a stronger depressive action than azathioprine in the same dose (the mean length of survival of the grafts was 16 ± 1.9 and 13.4 ± 0.62 days, respectively). In a dose of 20 mg/kg, KI-7 had a weaker immunodepressive effect.

The comparative study of the effect of azathioprine and KI-7 on regeneration in the rat liver showed that if used in the same doses they differed in their action on this process. For instance, KI-7 not only did not inhibit mitotic activity, like azathioprine, but actually stimulated it a little. This was shown as the earlier appearance of the first mitoses in the regeneating liver of the rats receiving KI-7 and also in the fact that the peak of mitotic activity occurred sooner and was higher than in the control animals receiving CMC alone: The

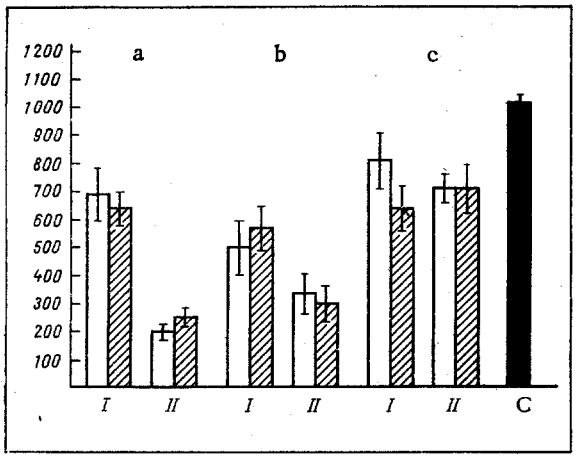

Fig. 1. Immunodepressive action of different doses of KI-7 and azathioprine on synthesis of antibodies against sheep's red blood cells depending on time of injection of compounds. Unshaded columns — KI-7; obliquely shaded columns — azathioprine; black column — control. Abscissa, doses of compounds: I) 500 mg/kg; II) 1000 mg/kg, given 1 day before (a), on day of (b), and 1 day after (c) immunization with sheep's red cells; ordinate, number of AFC per 10^7 spleen cells.

mitotic index in the experimental group 30 h after the operation was 28.9 ± 4.10 ‰, whereas in the control group the mitotic index reached its highest value, namely 22.5 ± 3.57 ‰, only after 48 h. When azathioprine was given the peak of mitotic activity occurred 72 h after partial hepatectomy and the mitotic index remained all the time below the control level of 15.4 ± 0.93 ‰.

As regards DNA synthesis, here also differences were observed in the action of the two immunodepressants. In rats receiving KI-7, a lower percentage of hepatocytes incorporating the label (18.4 ± 3.0) was observed 22 h after resection than in the regenerating liver of the control animals not receiving the immunodepressants (30.0 ± 2.5), although the value of ILN was higher than that obtained for the regenerating liver of the rats receiving azathioprine (8.0 ± 1.3%; P = 0.006). No differences in the action of azathioprine and KI-7 on the number of binuclear and hypertrophied cells in the regenerating liver could be observed.

The new chemical agent KI-7 is thus less toxic than azathioprine, depresses the synthesis of antibodies against sheep's red blood cells in mice if injected the day before or on the day of immunization, and also considerably prolongs the survival of skin allografts in rabbits compared with the control.

It has also been shown that when the system of immunogenesis is inhibited regeneration of the liver nevertheless takes place in rats, but the relation between the processes of proliferation and cellular hypertrophy becomes different from those during regeneration under ordinary conditions, and they depend on the immunodepressants used. Unlike azathioprine, for instance, KI-7 not only did not inhibit the mitotic activity of the regenerating liver, but actually stimulated it a little.

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