EFFECT OF Rhodiola EXTRACT ON CYTOTOXIC ACTIVITY OF NATURAL KILLER CELLS OF THE LIVER, SPLEEN, LUNGS, AND SMALL INTESTINE OF PARTIALLY HEPATECTOMIZED RATS

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One factor involved in the regulation of reparative regeneration and neoplastic proliferation is the system of natural killer (NK) cells. Inhibition of development of experimental tumors during repair of a number of organs (liver, kidneys) after their partial resection has been shown to be largely associated with the increased functional activity of these cells [1]. During stimulation of regeneration of the resected liver of rats with Pliss lymphosarcoma, inhibition of proliferation of tumor cells and metastases is significantly enhanced by the adapteogenic preparation Rhodiola extract (RE) [7]. This effect may be associated with increased cytotoxic activity, in the first place, of NK cells, but also of macrophages – cell populations located not only in the liver, but also in tissues of the spleen, lungs, and small intestine [8, 10]. The aim of the present investigation was to study the selective action of RE on functional activity of NK cells in these tissues in the early (1-2 days) and late (10 days) stages after partial hepatectomy (PHE) in rats, when functional activity of the liver macrophages is minimal [6].

EXPERIMENTAL METHOD

Experiments were carried out on 200 male Wistar rats weighing 200-220 g, from the "Rassvet" Nursery, Tomsk. The animals were divided into the following groups: 1) control; 2) rats receiving the pharmacopoeial preparation of RE (liquid extract of Rhodiola) during the morning for 12 days in a dose of 0.5 ml/kg orally, daily; 3) animals undergoing PHE (two-thirds of the organ) under ether anesthesia by the method in [9], and 4) rats undergoing PHE after preliminary administration of RE, starting 2 days before the operation. Functional activity of NK cells was assessed 1, 2, and 10 days after PHE, and cells of human erythromyeloid leukemia K-562, labeled with ^{51}Cr, were used as the target cells. The target cells were incubated in the presence of 3.7 MBq/ml of $Na_2{}^{51}Cr_2O_7$ in medium RPMI-1640 (Serva, West Germany) with the addition of 10% embryonic calf serum (Flow Laboratories, England) in an atmosphere of 5% CO_2 for 1 h at 37°c. At the end of incubation the cells were washed with medium 199 and diluted with Hanks' solution to a final concentration of $2 \cdot 10^6$ cells/ml. The resulting suspension was injected intravenously into the rats in a volume of 0.1 ml/200 g. The animals were killed by decapitation after 0.5, 1, 2, and 4 h and incorporation of the label into tissue samples of the liver, spleen, intestinal mucosa, and lungs was determined with the aid of a counter (Tracor Analytic, USA), and the result expressed per gram mass of the organ. The half-elimination time ($T_{1/2}$) of the label was calculated by "Élektronika MS 0585" computer [5]. NK-cell activity was determined as $1/T_{1/2}$ taking values of this parameter in the control for each tissue as 100%. The results were subjected to statistical analysis by the nonparametric Wilcoxon–Mann–Whitney test [3].

EXPERIMENTAL RESULTS

The results are given in Table 1. In animals receiving RE, NK-cell activity in the tissues of the liver and lungs was virtually unchanged throughout the period of the experiment, but in the lungs values of this parameter were lower than in the control. The level

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TABLE 1. Parameters of Cytotoxic Activity of NK Cells of the Rat Liver, Spleen, Lungs, and Small Intestine at Various Times after PHE and against a Background of a Course of Injections of RE (percent relative to control)

Group	Liver			Spleen			Lungs			Small intestine		
	1	2	10	1	2	10	1	2	10	1	2	10
1		100			100			100			100	
2	95	119	79	78	115	322(1)	58	73	63	128	212(1)	88
3	39(1)	82	180(1)	130	142	83	54	135	322(1)	51	100	48
4	23(3)	123	100(2)	161(3)	189	63(3)	88	435(2,3)	100(2)	75	114	50

Legend. Numbers in parentheses denote numbers of groups compared, differences from values of which are significant at the $p_{\mu} \leq 0.05$ level.

of NK-cell activity in the splenic tissue rose significantly toward the end of the experiment, but in the small intestine it did so after five injections of RE.

After PHE, NK-cell activity fell in all organs except the spleen; this process was most marked, moreover, in the liver tissue. Values of this parameter did not differ from the control as late as on the 2nd day after the operation.

PHE, performed against the background of RE administration, led to a marked decrease in NK-cell activity only in the liver tissue on the 1st day after the operation, but by the 2nd day the value of this parameter was the same as in the control. It was at these times that the highest values of activity of NK cells located in the lung tissue, and also the spleen, were observed, and where NK-cell activity fell toward the end of the experiment. The time course of NK-cell activity in tissue of the small intestine was the same.

There is evidence that adaptogens, including the Rhodiola preparation rhodoside, are unable to enhance the nonspecific immunobiological resistance of the recipient [4]. However, the results of the present investigations lead to the conclusion that RE has a differential effect on functional activity of one component of this system, namely NK cells, and increases their effectiveness in some tissues (small intestine, spleen) while reducing it in others (lungs). The cause of the increase in NK-cell activity in the small intestine may be to a certain degree its local effect, connected with the direct action of the preparation on the mucosa when administered by the oral route. We know that if RE is given five times (as in the present investigation) it significantly stimulates the proliferative activity of the epithelial cells of the small intestine [2]. It can accordingly be tentatively suggested that in the experiment described, the preparation exhibits a similar effect and increases the activity of NK cells located in the small intestine. The possibility cannot be ruled out that the increase in functional activity of NK cells of the spleen by RE toward the end of the experiment was due to the prlonged and stable action of the preparation on that organ – the main source of NK cells in the body.

Any stressor realizes its action on the living organism by inhibiting functional activity of NK cells located in different lymphoid organs, and in the case of animals undergoing PHE, this effect becomes most marked in relation to NK cells in the liver [11]. We found a similar rule in rats undergoing PHE, in all organs studied except the spleen, in which NK-cell activity is maintained at a high level throughout the experiment. Possibly a definite role in the elucidation of this phenomenon may be played by an effect of redistribution of effector cells.

If PHE is performed against a background of RE administration the stressor effect of the operation is significantly reduced, and this was accompanied by maintenance of NK-cell activity in all organs except the liver. The effect of a course of the preparation, leading to stimulation of reparative regeneration of the injured liver, can also explain the earlier recovery of NK-cell activity, i.e., a shift of the peak of their maximal effectiveness to earlier times, and the normalization of this parameter toward the end of the experiment.

It can thus be concluded that the use of RE or performance of PHE on animals has effects that differ in direction and intensity relative to activity of NK-cells located in the liver, spleen, lungs, and small intestine. If PHE is carried out against a background of a course of RE, which exhibits stress-regulating activity and increases the efficacy of postoperative regeneration of the liver tissue, it will promote the earlier recovery of the efficacy of NK cells and increase their activity, mainly in the lung tissue.

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DIPEPTIDYLPEPTIDASE-4 AS A SURFACE MARKER OF HUMAN NATURAL KILLER CELLS

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Dipeptidylpeptidase-4 (DP-4) is a serine protease which catalyzes removal of dipeptides from the N-terminal residue of oligo- or polypeptides, if a proline, hydroxyproline, or alanine residue occupies the second position. DP-4 is found in virtually all organs and systems of the human body [6]. It is possible that DP-4 performs different functions in different situations in the body. For instance, DP-4 localized on the surface of lymphocytes is evidently connected wtih processes of lymphocyte proliferation [9]. A high level of interleukin-2 (IL-2) production by cells carrying DP-4 on their surface has been reported, when IL-2 production in populations of DP-4 cells is very low [10]. It is possible that DP-4 is a marker of IL-2-producing cells.

Natural killer (NK) cells constitute a special subpopulation of lymphocytes capable of producing lysis of cells of many autologous and allogeneic tumors, and also of nontumor, virus-infected cells without preliminary immunization and without recognition of antigens of the histocompatibility complex [7]. Besides the cytotoxic function, their ability to secrete lymphokines also has been described: for example, B-cell growth factor (BCGF), Il-2, interferon (IFN), colony stimulating factor (CSF) [8, 14].

Morphologically NK cells form a population of large granular lymphocytes (LGL) [4, 13]. They carry on their surface markers such as the Fc-receptor for immunoglobulin G ($Fc_\gamma R_1$ CD16.Leu11), HNK-1 (Leu7), and a receptor for the third component of complement (CR3, KM1, CD11) [13, 14].

The aim of the investigation was to study DP-4 activity on the surface of NK cells from human peripheral blood.

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