INDUCTION OF CYTOTOXIC ACTION BY RIFAMYCIN IN A SYSTEM OF NORMAL ALLOGENEIC LYMPHOCYTES–TARGET CELLS IN VITRO

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Normal mouse lymphocytes incubated with rifamycin (100 μg/ml) for 45 min at 37°C and washed 3 times to remove the antibiotic after contact destroyed allogeneic tumor target cells. Increased toxicity of normal lymphocyte also was observed during combined incubation of lymphocytes and allogeneic target cells in the presence of antibiotics in doses nontoxic for the cells (10 μg/ml). In the study of the action of rifamycin on the cytotoxic effect of the immune lymphocytes in some experiments this effect was very slightly weakened.

In the experimental search for and selection of substances with possible antitumor activity guidance is usually taken from the degree of inhibition of growth of transplantable tumors after direct contact between the tested agent and the tumor cell [1]. Yet as has been shown previously, some natural compounds and synthetic chemotherapeutic substances (PHA, methotrexate, actinomycin D, mitomycin C, interferon, etc.) [2, 3, 7] may inhibit tumor growth not directly (or not only directly), but also through induction of cytotoxic action in normal lymphocytes. As a result of this, the study of the ability of newly obtained hemotherapeutic preparations, and also of others already used in practice, assumes importance on its own account.

The possibility of activation of lymphocytes by rifamycin in a system of lymphocytes–tumor target cells and also the action of rifamycin on the cytotoxicity of immune lymphocytes toward tumor target cells were studied.

EXPERIMENTAL METHOD

The experimental method was described earlier [2] and was based on the model of transplantation immunity in vitro suggested by Rosenau and Moon [10].

TABLE 1. Cytotoxic Action of Normal Allogeneic Lymphocytes in the Presence of Rifamycin in a Dose of 10 μg/ml (M ± m)

Experiment No.	Donor mice	No. of living L-cells 48 h after incubation (×1000)				
		normal lymphocytes	P	normal lymphocytes + rifamycin 10 μg/ml	rifamycin 10 μg/ml	medium No. 199 (control)
29	C57BL/6j	124±6,5	<0,01	98±1,2	157±8,4	154±13,5
31	BALB/C	110±8,2	>0,05	90±5,3	171±7,5	122±3,9
101	C57BL/6j	124±6,5	<0,01	93±4,0	164±5,6	154±13,5
105	C57BL/6j	78±6,8	>0,05	66±2,9	84±4,3	97±7,7
119	C57BL/6j	566±11,5	<0,001	224±33,5	590±64	574±24,7
32	BALB/Cj	82±6,0		93±3,0	99±7,4	87±6,3
104	C57BL/6j	209±15,0		222±16,2	183±8,8	192±14,0

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TABLE 2. Cytotoxic Action of Normal Allogeneic Lymphocytes Preincubated with Rifamycin in a Dose of 100 μg/ml at 37°C for 45 min and then Washed 3 Times (M ± m)

Experiment No.	No. of living L-cells 48 h after incubation (×1000)			
	normal lymphocytes	P	normal incubated lymphocytes	medium No. 199 (control
114	103±5,6	<0,05	83±6,2	138±12,6
116	169±15,6	>0,05	144±4,6	172±28,2
117	348±20,4	<0,01	265±12,5	357±71,0
118	226±16,0	<0,05	165±26,0	280±6,75
119	566±11,5	<0,01	349±44,5	574±27,4

Rifamycin (Lawson) was used in the experiments as a 0.1% solution in medium No. 199. The target cells were a 24-h monolayer of L-cells grown in glass tubes on medium No. 199 with 10% inactivated bovine serum. L-cells are a transplantable strain of fibroblasts obtained from C3H mice.

The donors of the normal and immune lymphocytes were BALB/c and C57BL/6j mice. Lymphocytes were taken only from the lymph glands. The technique of immunization of the mice with L-cells and of obtaining normal and immune lymphocytes was described earlier [3].

Lymphocytes were added to target cells after removal of the nutrient medium in a proportion of 4 million cells per tube in 1 ml medium No. 199 without serum. The medium of the experimental groups contained rifamycin in doses nontoxic for L-cells. During work with higher doses of the antibiotic, inhibiting growth of the L-cells, the lymphocytes were incubated in the presence of the L-cells for 45 min at 37°C and then washed 3 times.

The experimental results were read 48 h after addition of the lymphocytes as the number of living target cells. For this purpose the cells were stained with a mixture of eosin and trypan blue, as described earlier [4].

EXPERIMENTAL RESULTS

The toxicity of rifamycin for a 24-h monolayer of L-cells and for lymphocytes was determined in a preliminary series of experiments. A dose of 10 μg/ml did not reduce the number of surviving target cells compared with the control but doses of 50 and 100 μg/ml led to death of approximately 50 and 70% of L-cells respectively 48 h after combined incubation.

The toxicity of rifamycin for lymphocytes was exhibited only in a dose of 100 μg/kg, i.e., lymphocytes were more resistant to the action of the antibiotic than L-cells.

In each of the subsequent experiments a parallel control of the toxicity of the preparation against target cells was set up. Rifamycin was used in the experiments in doses of 10 and 100 μg/ml (Tables 1-3).

The results in Table 1 show that lymphocytes in the presence of rifamycin gave a marked cytotoxic action in 5 of the 7 experiments. In 3 experiments (Nos. 29, 101, 119) the difference between the experimental and control groups was statistically significant. In experiments No. 31 and 105 this difference was not significant. In one of these experiments (No. 31) rifamycin, added without lymphocytes, stimulated growth of the L-cells compared with the control and this may have been the cause of the very low level of cytotoxic action of the lymphocytes. In another experiment (No. 105) normal lymphocytes themselves, without rifamycin, were cytotoxic for the L-cells; in this experiment, moreover, rifamycin also by itself, without lymphocytes, inhibited growth of the target cells. In two experiments (Nos. 32 and 104) the lymphocytes had no cytotoxic action.

To rule out the direct action of rifamycin on target cells in the next experiments lymphocytes incubated with 100 μg/ml rifamycin for 45 min at 37°C and washed to remove the antibiotic were added to L-cells. In these experiments the lymphocytes treated with the antibiotic had a marked destructive action on the cells. In 4 of the 5 experiments the difference was statistically significant (Table 2).

TABLE 3. Cytotoxic Action of Immune Allogeneic Lymphocytes in the Presence of 10 μg/ml Rifamycin (M ± m)

Experiment No.	No. of living L-cells 48 h after incubation (×1000)				
	immune lymphocytes	P	immune lymphocytes in the presence of rifamycin 10 μg/ml	rifamycin 10 μg/ml	medium No. 199 (control)
32	43±9,4	>0,05	59±7,0	99±16,4	88±6,2
111	4±1	>0,05	6±2,2	207±17,6	173±11,0
113	29±3,1	<0,01	42±2,3	65±5,4	64±8,0
117	146±23,5	>0,05	170±5,6	350±33,0	357±70,0
118	25±3,9	>0,05	35±11,0	122±20,0	280±67,5
119	195±13,4		127±16,9	390±63,5	574±27,4

The study of the effect of rifamycin on the cytotoxic action of the immune lymphocytes showed that the number of surviving target cells after the addition of the antibiotic was a little higher than in the control. In one experiment this difference was statistically significant (Table 3); i.e., rifamycin slightly reduced the cytotoxic action of the immune lymphocytes.

Rifamycin is a substance receiving the closest study at the present time. It is known as a specific inhibitor of transcriptases [6, 11, 13]. The evidence of its action on normal and transformed cells in vitro and on the actual process of transformation of cells in culture by oncogenic viruses is extremely contradictory [5, 8, 9, 12, 14]. A more detailed study of the phenomenon of induction of the cytotoxic action of normal mouse lymphocytes by rifamycin could help to elucidate these contradictions and also to clear up the enigma of the mechanism of activation of lymphocytes as a whole.

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