A Trial Of New RL-Series Preparations In Normal Hemopoiesis Correction

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Citation

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Abstract

We have conducted experimental and clinical approbation of two preparations coded RL-175 and RL-S from the new generation of synthesized active substances. Their action is directed to increase the mechanisms of the hemopoietic growth factors _expression. These preparations affect the stem cells and enhance their proliferation and differentiation mechanisms. They regulate the genes p53 and bcl-2 activity and thus provide for the apoptosis mechanism starting in the onthogenesis.
During the correction of the normal hemopoiesis in patients with otherwise poor prognosis ... especially those with chronic and sharp variants of leukemia in the expanded and terminal stages without further remission - we proved the high efficiency of RL-175 and RL-S preparations by their availability, cost and effect over the well-known geno-engineering bio preparations (human recombinant- erythropoietin, trombopoetine, interleukine (IL-1), (IL-2), IL-6 and others.

INTRODUCTION

Since the time of cytostatic and radical anti-tumor treatment, a compelling need arised for preparations that stimulate and regulate normal hemopoiesis. Over the decades, the problem needs even more energetic research due to the high morbidity associated with leukemia. Gene-engineering and microbiologic methods appear to be extremely fruitful in production of anti-tumor bio-preparations that demonstrated a stimulating effect on the hemopoiesis granulocytic germ [1,2,3,4]. However, wide applications of the human recombinant erythropoietin (r-Hu-EPO), interleukin-1beta (IL-1), granulocytic-macrophageal factor (GM-CSF), granulocytic colony-making factor (G-CSF) and others, as well as thymus peptides clone-preparations [5] for anemia correction are restricted due to high cost and sometimes poor efficiency in correction of hematologic toxicity appearing under intensive chemotherapy [6, 7].

Leukin (IL-1) and leukomax (molgramostine), and GM-CMF as an active component and also the use of peripheral truncal cells (mobilized by CSF after the chemotherapy course) behave like stimulators and protectors of leukopoiesis under postcytostatic myelodepression in patients with lymphoprolipherative diseases. However, they do not entirely correspond to E. Frei's phenomenon stating (1979) that tumoricidic effect increases in proportion with its concentration in cultural medium [6].

In this report we present the results of the RL-175 and RL-S's influence on the hemopoietic processes of healthy and ill (hemablastosis) animals (rats and cows) and also hopeless volunteers with system diseases at extended or terminal stages. The investigated compounds surpass the above-mentioned bio-preparations in availability, normal hemopoiesis correcting effect and also in their protecting and leuko-stimulating influence in patients suffering from hemopoiesis depression.

MATERIALS AND PROCEDURES

Experiments included 20 rats and 73 cows which were both healthy and diseased animals. The tested animals were of an equal age and weight, and of the same breed of species. A total of 73 cows were involved in the experiments. From this group 23 cows were hemoblastosis cases on the basis of hematological data obtained in accordance with the Instruction for cattle leukosis control, issued by the Russian veterinary authorities in 1973. In this group of animals, the leukocyte count in 1 mm^3 of blood was 18 000-24 000 and the lymphocyte content was 80-85%. The leukocyte sedimentation reaction was positive at more than 70% of these cows. The test group designated as VII, consisted of 13 animals. In this group RL-S was injected into jugular veins in the form of 0.9% isotonic solution. The total dose was 160 mg (three daily injections, each of 53 mg RL-S in 5 ml of physiological salt solution). In the control group designated

as VI consisting of 10 animals, only 5 ml of physiological salt solution were injected in the same sequence as in the Group VII.

The experiments were applied to 50 healthy cows divided into five groups, each consisting of ten animals. Group I was the control. Groups II-V were test groups. The control animals were only given daily injections of 5 ml of physiological salt solution. The test animals were intravenous injections of different RL-S doses dissolved in 5 ml of physiological salt solution. The effect of different RL-S doses on hemoglobin (Hb), erytrocytes (Ery) and leukocytes (Leuk) in the peripheral blood of diseased (Groups VI and VII) and healthy (Groups I-V) are presented in the Table 1 and Figure 1. Long-term effects of RL-S on the hemopoiesis of the animals involved in the experiments are presented in the Table 2.

Figure 1

Table 1: Effects of Different RL-S Doses on Hemoglobin content and Erythrocyte and Leukocyte Counts in Blood of Healthy (Groups I-V) and Diseased

(Groups VI-VII) Animals (p<0,0001)*

Single dose per head	Injections number	Total dose (mg)	Hb (g/l) M±m	Ery, $*10^{12}$ / liter M±m	Leuk, $*10^{9}$ / liter M±m
Control (I)	3(6)	-	98,0±0,6	6,4±0,5	6,9±0,5
28 (II)	3	84	114,0±1,0	4,0±0,4	6,9±0,4
28 (III)	6	168	126,0±0,9	9,6±1,5	9,1±1,3
53 (IV)	3	159	129,0±0,7	9,8±1,0	6,6±1,8
53 (V)	6	318	89,0±1,6	7,5±1,8	10,0±1,3
Control (VI)	3	-	92,0±0,6	6,9±0,5	21,0±3,1
53 (VII)	1	53	80,0±0,6	6,1±0,7	17,7±1,7
53 (VII)	2	106	112,0±0,9 142,0±0,8	6,6±0,9	9,8±1,1
53 (VII)	3	159		10,2±1,0	6,7±0,8
Normal values	-	-	123,0-93,0	7,5-4,5	9,5-6,5

* Hb, Ery and Leuk indices against RL-175 effect on the animals were close to control ones (Tables 1 and 2)

Figure 2

Table 2: Hemoprofile of Randomly Chosen Healthy and Leukotic Cows 120 Days after the Last Injection of RL-S

Animals No	Hb, g/l	Ery, $*10^{12}$ /l M±m	Leuk, $*10^{9}$ /l M±m	Eos, % M±m	Neut, %		Lymph, %	Mono, %	Color index
					Rodlike nucleus	Segmented Nucleid			
8675	120 (103)	9,4 (3,4)	6,8 (4,5)	5,0 (1,0)	1,5	22	69	2,0	0,77
1104	140 (117)	10,3 (4,4)	6,4 (6,4)	3.0 (0,5)	1,0	17	78	3,0	0,82
8163	113 (107)	8,7 (3,8)	7,8 (8,7)	3,5 (1,5)	1,0	11	83	1,0	0,78
8414	122 (110)	9,3 (3,2)	7,5 (5,4)	2,0 (1,0)	1,5	46	47	4,5	0,79
8182	120 (108)	9,3 (3,7)	7,2 (9,0)	2,0 (1,0)	1,0	14	82	1,5	0,77
1550	110 (102)	8,6 (3,6)	7,9 (7,0)	3,0 (0,5)	1,0	14	80	3,0	0,77
8452	113 (103)	8,4 (3,3)	8,0 (4,0)	0,5 (0)	1,5	24	70	1,0	0,81
8135	130 (102)	9,2 (7,1)	7,2 (18,6)	5,0 (0)	4,0	38	51	4,5	0,85
8369	142 (104)	10,2 (7,4)	6,7 (12,4)	4,0 (0)	1,0	14	78	3,0	0,84
1713	112 (92)	6,8 (6,3)	10,3 (24,2)	3,0 (0)	1,0	20	63	1,0	0,99
Normal values	123-93	7,5-4,5	9,5-6,5	13-1	12-1	40-10	70-40	13-1	1,3

(figures in brackets refer to the condition of animals prior to the experiment)*

* Prior to the experiment lymphocyte index varied from 80 to 85%, neutrophile index with segmented nuclei varied from 1 to 10%, and monocyte index varied from 0 to 1%.

Experiments were further made on female Vistar rats aged 6-9 months and weighing 120-150 g. A total of 20 rats were involved in the experiments. They were divided into two groups, each consisting of 10 animals and designated as VIII and IX respectively. Group VIII was the control and Group IX was the test group. The animals of Group IX were given one daily intravenous injection of 8 mg of RL-S in 1 ml of physiological salt solution per day during three days. the control animals of Group VIII were given one injection of 1 ml of physiological salt solution per day during three days.

In all the cow and rat groups, blood samples were taken in the morning on an empty stomach. In both the control and test groups, the animals were given normal rations and were kept in normal veterinary and zootechnical conditions. All requirements, which are required in the work with laboratory animals were observed.

PATIENTS AND METHODS

There were 20 patients involved 4 males and 16 females, aged from 2 to 65 years old and an average age of 27 years with the following morphologically verified diagnoses:

1. acute myeloleukemia – 1 case
2. myelomonoblastic leukemia – 1;
3. acute lymphoblastic leukemia – 3;
4. acute leukemia (non-typed variant) – 2;
5. chronic myeloleukemia – 3;
6. acute undifferentiated leukemia – 1;

7. acute myelomonoblastic leukemia – 4;
8. prolymphocytic lymphoma – 1;
9. chronic lymphoblastic leukemia – 1;
10. lymphogranulomatosis – 1;
11. nonhodgkin's lymphoma- 2.

The experiments were then extended to 20 hopeless patients of different age and sex, all of them volunteers with scrupulously documented cancer cases. Prior to the experiments all the patients had undergone complete courses of up-to-date chemotherapy. In each of the 20 cases chemotherapy courses resulted either in very little or no effect.

They also suffered systemic diseases, namely acute leukemia and related diseases, as well as chronic lymphoid and myeloid leukemia in advanced or terminal stages (the blast crisis).

At the hopeless volunteers', approbation RL-175 preparation was the most studied drug in terms of physiological parameters, though it didn't take any special effect on the hematopoietic system in relation to control during the experiments (Tables I and II).

RL-175 was administered daily to all the patients orally on an empty stomach for 30 days. A daily dose of the preparation was 5 mg dissolved in 10 ml of fruit syrup or physiological salt solution.

In the case of tumoral diseases of hematopoietic and lymphoid tissues, RL-175 is regarded as fully effective if it eliminates all clinical symptoms of the disease, normalizes the state of the blood and reduces the blast content in the bone marrow to no more than 5% at the normal cell count. All information concerning the tests including patients' condition and tumoricidal effect of RL-175 is presented in the Table 3.

Figure 3

Table 3: Hemopoiesis Before and After 30-Day RL-175 Treatment (values listed as numerator and denominator, respectively)

Disease	Sex, age (years)	ESR, mm/hr	Hb, g/l	Ery, *10^{12}/l	Color index	Leuk *10^{9}/l	Thro *10^{11}/l	Baso, %	Eos, %
Acute myelomonoblastic leukosis	female 16	6/3	120/130	4,18/4,04	0,9/0,9	1,1/5,3	0,418/3,1	0/3	0/4
Chronic myeloleukosis	male 41	3/3	140/152	3,8/5,0	1,0/0,9	111,0/20,1	2,47/5,7	0/1	1/2
Prolymphocytic lymphoma	male 61	16/5	100/113	3,4/4,14	0,8/0,8	50,6/9,0	1,9/2,3	0/2	2/5
Chronic lympholeukosis	male 65	12/2	121/142	4,35/4,5	0,85/0,95	30/9,4	1,7/2,25	0/2	0/4
Acute lymphoblastic leukosis	male 6	37/7	50/96	1,62/3,1	-	4,3/4,3	0/2,2	0/0	0/6
Acute leukosis (not typed)	female 13	20/5	60/130	2,3/4,0	0,8/0,8	2,7/4,2	0,60/4,2	0/0	0/4
Acute myeloblastic leukosis	Female 16	65/35	83/100	2.8/3.3	0,9/0,9	1,1/3,3	0/1.35	0/0	0/1

Disease	Neut, %				Blasts, %*	Lymph, %	Mono, %
	Myelocytes	Young	Rodlike nuclei	Segmentel nuclei			
Acute myelomonoblastic leukosis	0/0	0/0	1/6	15/63	83/1	1/25	0/2
Chronic myeloleukosis	17/11	8/4	26/13	38/51	4/1	6/18	2/1
Prolymphocytic lymphoma	0/0	0/0	4/10	13/17	70/4	10/70	2/4
Chronic lympholeukosis	0/0	0/0	2/3	4/39	1/0	87/52	2/3
Acute lymphoblastic leukosis	0/0	0/1	0/1	15/52	4(100)/4(5.8)	77/33	1/11
Acute leukosis (not typed)	0/0	0/0	1/5	10/45	67/0	22/45	0/2
Acute myelomonoblastic leukosis	0/0	0/0	2/5	15/68	71/5	12/20	0/1

* In the "Blasts" column, figures in brackets refer to the blast index in the bone marrow whereas the figure before the brackets refers to the blast index in the peripheral blood.

RL-175 and RL-S are heteroaromatic compounds. Their physico-chemical and biological properties are described in reference [8]. They can be synthesized from readily available raw materials. In rats, the LD16 of this preparation is 1.200 mg per 100 g of body weight. Its chemical structure is stable enough for storage. The RL-175 and RL-S treatment course costs 100 US dollar.

Clinical and hematological studies of RL-175 and RL-S were conducted in accordance with standard methods.

STATISTICAL ANALYSIS

The difference between control groups and treatment groups was determined using Student's t test.

Hopeless volunteers, who gave written agreements, were included in the study. Ethic norms of patient privacy in correspondence with the principles of the Declaration of Helsinki (1964) and 1995 (as revised in Edinburgh, 2000) were preserved.

RESULTS

Concerning the effect of the RL-S on hemoglobin, erythrocyte and leukocyte counts in the peripheral blood of

cows, one can conclude from the Table 1 and Diagram 1that the optimum single dose is 53 mg per head and the maximum number of injections is three. The resultant total dose of 159 g reduces the leukocyte count to the normal physiological level, considerably increasing hemoglobin level and erythrocyte index. Meanwhile the results of three 53 mg intravenous injections (see Fig. 1) are as follows: in the test group hemoglobin level is 156±0.6 g/l, erythrocyte index is $9.8*10^{12}$ per liter, leukocyte index – $8.3*10^{9}$ per liter, whereas in the control group hemoglobin level is 102±0.5 g/l, the erythrocyte index is $6.8*10^{12}$ per liter and leukocyte index – $7.4*10^{9}$ (P>0.001) per liter.

In the Groups I-IV subsequent single doses of more than 60 mg rapidly reduced hemoglobin level and erythrocyte index, simultaneously increasing leukocyte index. This was followed by administering optimum doses to the animals of the Groups I-V. As a result, leukocyte index was brought back to normal with significant increase of hemoglobin level and erythrocyte index.

It should be noted that in addition to 20-60% increase in hemoglobin level and erythrocyte index one can selectively control hemopoietic processes over a broad range of process parameter values by varying the RL-S dosage (see Tables 1 and 2).

The RL-S effect is even more striking in rats. In the Group IX three injections of RL-S almost doubled hemoglobin level and erythrocyte index, raising them respectively to 174±1.0 g/l and $7.5±0.15*10^{12}$ per liter. The color index in this group was 1.7 to 1.0, leukocyte index – $6.0±0.2*10^{12}$ per liter. In the Group VIII – the control one, hemoglobin level was 104 ± 0.6 g/l, erythrocyte index was $3.16±0.2*10^{12}$ per liter, color index – 1.8 to 0.7, leukocyte index – $10.0±0.1*10^{9}$ per liter. High degree hemopoiesis persisted for a long period.

Table 3 presents selected clinical and hematological data on patients with pathological changes in hematopoietic and lymphoid tissues.

As understood from the Table 2, RL-175 intravenous injections rapidly normalize neutrophile, eosinophile, monocyte and lymphocyte indices. This indicates an improviement of the cellular composition in the bone marrow.

Table 3 and figure 2 present changes in hematological status of patients with diseased hematopoietic and lymphoid tissues. They demonstrate that the blast index is rapidly brought back to physiologically normal level. Normalization of hemopoiesis and of the leukocyte index is accompanied by marked increase in hemoglobin content, also in erythrocyte, thrombocyte, monocyte and segmentation nucleus index. These results showed that RL-175 is an effective stimulator of hematopoietic factors, independent of lymphocytic activity.

As seen from Table 3 and figure 2, RL-175 injections cause complete leukemia remission in terminal stages in about 60-90% cases.

DISCUSSION

RL-175 oral administration to humans and RL-S to animals with tumoral diseases of hematopoietic and lymphoid tissues (Tables 1-3, figures 1, 2) causes tumor regression in almost 100% cases and rapidly normalizes granulocyte (neutrophil and eosinophil) and monocyte blood indices. It almost completely activates lymphocytes. Leukocyte index gets normal as well. Hemoglobin content and erythrocyte index in some cases doubles compared to the control.

RL-175 has a broad spectrum of influence on the patients in extreme post-cytostatic myelodepression. For example, leukocyte number in the peripheral blood of the patient K.R. (*10, Fig. 2, chronic myeloleukemia, extended stage) had been $123*10^{9}$ per liter before and got $20*10^{9}$ after RL-175 injections. Before the RL-175 trial there were isolated cells of Philadelphia chromosome (Ph) found in myelogramma, and no Ph after the approbation.

One patient (chronic myeloleukemia, extended stage) had $310*10^{9}$ leukocytes per liter and 22% blast cells in his hemogram before the specific treatment, and $30*10^{9}$ per liter leukocytes and 4% blast cells after RL-175 treatment. No Ph chromosomes were found in the myelogram.

These patients had full hematological remission in the final period of RL-175 treatment. They led active way of life without any limits. The patient K.R. had been under observation for 10 years and the patient K.I. for 2 years.

In addition to stimulating the normal process of hemopoiesis after a course of up-to-date physio- and chemotherapy, RL-175 stimulates differentiation of blasts in myelodisplasia cases and normalizes proliferation of transformed stem cells (Fig. 2). All these processes are associated in literature [$_1$, $_2$] with regulation of gene _expression of the hemapoietic growth and colony-enhancing factors. It is known, for example, that GM-CSF, IL-3 and M-CSF genes are found in

Chromosome 5, whereas G-CSF is incorporated in Chromosome 17.

Beta-leukin (IL-1) and CSF exert compatible but not identical influence on hemopoietic cells [4, 7]. Myelo-depressed patients under IL-1 injections get an increase in leukocytes number from $1,2\pm0,1*10^9$ to $7,8\pm2,6*10^9$ per liter ($p>0,001$) and from $0,6\pm0,1*10^9$ to $3,2\pm0,1*10^9$ per liter respectively. The same indices under GM-CSF influence change from $1,4\pm0,1*10^9$ to $4,2\pm0,5*10^9$ per liter and granulocytes from $0,7\pm0,1*10^9$ to $3,0\pm0,4*10^9$ per liter ($p<0,001$).

Simultaneously beta-leukin exerts protecting effect concerning leukopoiesis damage, that it is completely extrinsic to hemopoietic CSF (GM- and G-CSF) [7].

CONCLUSIONS

We have conducted a comparative analysis of the granulocytopoiesis stimulating effect induced by preparations having a very simple chemical structure.

Leukostimulating effect of the RL-175 in relation to myelodepressive patients (n=10) is comparable by the proved leukocyte number increase from $1,4\pm0,1*10^9$ to $6,3\pm0,3*10^9$ per liter, ($p>0,05$), to IL-1 and GM-CSF effect.

The reverse of blast cells into normal cellular proliferation cycle (Figure 2) and quick normal hemopoiesis correction by more than 25% (Tables 1-3; Figure 1) prove more significant granulocytopoiesis stimulation by preparations of the RL series. This conclusion seems more reasonable when taking into account that the average life of 20 patients included in the study expanded by 2-4 years with a maximum of10 years.

Firm stabilization of the hemogram parameters of healthy and hemoblastosis cows within 120 days after the treatment course (Table 2, Figure 1) confirms RL-S's protecting effect on leukopoiesis. The discovered phenomenon of selective regulation of the blood components' levels (including leukocytes) depending on the doses of examined preparations, and also anti-tumoral effect of the combined chemo- and chemoradial therapy on the solid 4-year tumors [8] may prove higher efficiency of the RL-S and RL-175 preparations against IL-1, GM-CSF and other human recombinants.

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