Prospectives Of RL Preparation Use With The Aim Of Correction Of Anemia In Comparison With Recombinant Erythropoietine

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Citation

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Abstract

The information in this article is about a new generation of antianemic preparations of RL series synthesized by the author. It could be shown that the preparation of RL series (by the level of anemia correction) via induction of cellular genes transcription of the hemopoietic growth factors considerably overcame human urinary EPO and recombinant human rHu-EPO in experiment and clinic.

INTRODUCTION

In recent years, paramount investigations in research of stem cells and growth factors became the leitmotiv of all international Hematology conferences. Due to an increasingly transparent human genome new cellular and molecular technologies have emerged and newly discovered genes are being matched to assigned functions. But our knowledge about the genome is only the initial stage regarding causes of cell malignization because the activity of expressing genes [1] is under systemic control of human brain hormones according to the scheme [2]:

hypothalamus hypophisis gonades genes.

Already today, a huge gap in studies between the gene structure and its function has been revealing [3]. This gap will increase many times along with new discoveries of thousands of new brain genes. This fact requires serious reevaluation of traditional approaches to the strategy of fighting leukemia. This work is devoted to this topic.

EXPERIMENTAL AND CLINICAL PROCEDURES

86 cows of different age (4-8 years old) and breed were used. According to the veterinarian service data, 20 animals suffered hematoblastosis and 16 brucellosis. 9 groups were created (Table 1).

Figure 1

Table 1: The Changing of hemoglobin (Hb), erythrocytes (ery) and leukocytes (leuk) in peripheral animal blood with introducing to them different doses of RL-S (p 0,05).

Animal, its physiological state	Dose of RL-S g/l on a head x 10^{-3}	A number of injections	Total dose g/l x 10^{-3}	Hb g/l	Ery x10^{12}/L	Leuk x10^{9}/L	% of increasing	
							Hb	Ery
Healthy rats (n = 20); Controlled (n = 10); Experimental (n = 10).	- 0,8	- 3	- 2,4	104±6,0 174±1,0	3,16±0,2 7,5±1,5	10,0±0,1 6,0±0,2	67,3	137,4
Healthy cows (n = 50); Controlled (n = 10); Experimental in n=10 every series by n=10 n=10 n=10	- 2,8 2,8 5,3 5,3	- 3 6 3 6	- 8,4 16,8 15,9 31,8	98±6,0 114±10,0 126±9,0 129±7,0 89±16,0	6,4±0,5 4,0±0,4 9,6±1,5 9,8±1,0 7,5±1,8	6,9±0,5 6,9±0,4 9,1±1,3 6,6±1,8 10,0±1,3	31,6	53,1
Cows ill with brucellosis (n = 16); Controlled (n = 6); Experimental (n=10)	- 5,3	- 3	- 15,9	102±9,4 156±10,2	6,8±0,6 9,8±0,6	7,4±1,5 8,3±1,3	53,0	44,0
Hemotoblasted cows: Controlled (n = 10); Experimental (n = 10).	- 5,3	- 3	- 15,9	98±6,6 142±8,0	6,9±0,5 10,2±1,0	21,0±3,1 6,7±0,8	45,0	48,0

* Preparation was introduced intravenously during 7 days.

Healthy and sick cows were chosen according to the breed, age and morbidity level. The animals were held in farms of the North Ossetia-Alania republic (Russia) according to zootechnic and veterinerian conditions and feeding. The experiments and observations of the cows were conducted in a timeframe of 7 months (time of observation). After the experiments, the animals were examined weekly.

Two groups (control and experimental) of healthy 10 wister female rats of 120-150g each were also used.

Preparation RL-S was injected to the animals intravenously with a 0,9 % isotonic solution: rats were dosed three times

by 0,8x10 $^{-5}$ g/l and cows were also dosed 3 times at different dose levels of RL-S (optimal dose 15,9x10 $^{-5}$ g/l per day) during 7 days.

A hematologic test was chosen as basic criteria for the selection of the optimal preparation dose. The results of are presented in the table 1. The animals were all treated according to the protocol clinico-hematological investigation.

The evaluations of the three cytologic actions of the preparations (RL-3, RL-175 and RL-S) were made by radiometric method [$_3$]. The results are presented in Fig.1 (a, b).

Administration of RL-175 preparations was given to 5 patients (4 males and 1 female; aged from 2 to 6 years) with severe lymphoblasted leukosis including blast crisis (myelograms showed 60-70% of blasts). RL-175 was daily administered orally on an empty stomach for 30 days. The daily dose of the preparation was 5 mg dissolved in 10 ml of fruit syrup or physiological salt solution.

RL-175 and RL-S are heteroaromatic compounds; their physicochemical and biological properties are described in [$_4$]. Clinical and hematological studies of RL-175 and RL-S were conducted in accordance with standard methods.

Clinical investigations on human subjects were carried out in generally accepted ethic and moral limits in accordance with declaration of Helsinki (1964) and 1995 (as revised in Edinburgh, 200).

STATISTICAL ANALYSIS

The difference between control groups and treating groups was determined using Student`s t test.

RESULTS

In the experiment, we showed that preparations under the code RL-S and RL-175 accelerated the oxidation of succinate by rat mitochondria (1,14x10 $^{-6}$ mole•c against 0,67x10 $^{-6}$ moleoc in control) by 60-80 % (P 0,01)[^{4}]. Qualitative thin-layer and gas-fluid chronomatography revealed increasing in vivo synthesis of ATP (up to 3 times compared to control indices) in skeletal and cardiac muscles of rats fed with RL-S and RL-175.

Veritable free energy (G) of ATP hydrolysis in pointed muscles cells (by concentration of ATP, ADP and P_i correspondingly 40; 0,93 and 8,05 MM and significance pH 7,0 and 25 C) did not increase. G discharged at hydrolysis of energetic value in intact erythrocytes, muscle and rat s liver [$_5$]:

Figure 2

$$\Delta G = \Delta G^{01} + 2{,}303RT \lg \frac{[ADP]\cdot[Pi]}{[ATP]} = -51{,}9kj/mole,$$

where ΔG^{01} – standard free energy; R – gas constant; T – absolute temperature and Pi – phosphoric acid.

With the account that synthesis of one molecule ATP from ADP and Pi is spent in standard thermodynamic conditions 30,5 kj/mole [^{5}], than difference in - 21,4kj/mol comprises the standard energy (G ^{01}) of displacing the preparations RL of mentioned above balanced system (pKa) in the pool of multifermental complex of respiratory chain in 10 ^{10} times regarding of balanced (pK a) uninvolved spontaneous passing of molecule A into B pK_a = - 1,15x10 $^{-3}$ [^{5}].

Figure 3

$$pKa = \frac{[B]\cdot[ATP]}{[A]\cdot[ADP]\cdot[Pi]} = 0{,}28x10^7$$

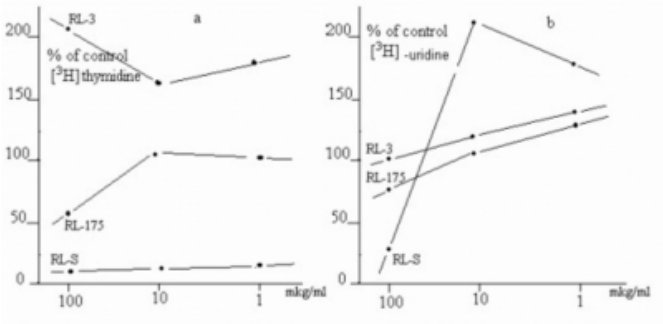
where for A spontaneously turning into B by G ^{01} = -21,4b Kj/mole pKa = 5,62x10 ^{3} , ratio [ATP]/[ADP][Pi] is equal to 500 approximately.

Apparently under the influence of RL in 3-key divisions of the respiratory chain not three but four molecules are synthesized. With the account of this standard balance pK_a in the chain is being displaced 10 ^{40} times what is considerably higher than in control physiological norm 10 ^{24} times [^{5}].

Structural dependence of the investigated compounds on the synthesis of DNA and RNA (based on inclusions of [^{3} H] – thymidine on [^{3} H] – uridin into the cell of human ovarian carcinoma $CaO_{}$ line) was revealed. For example, under the RL-3 influence synthesis of DNA decreased two times when the dose of preparation was 100 mkg/ml (fig 1a and b). This effect of RL-3 on the synthesis DNA is persisting even in lower concentrations.

Figure 4

Fig. 1. Dependence of including [3 H] - thymidine and [3 H] - uridine into the cell of human ovarian carcinoma line on the concentration of preparations of RL series (a, b) as well, as increasing of concentration of Hb in 5 patients with ALL and chronic kidney insufficiency from dose RL-175 (c).



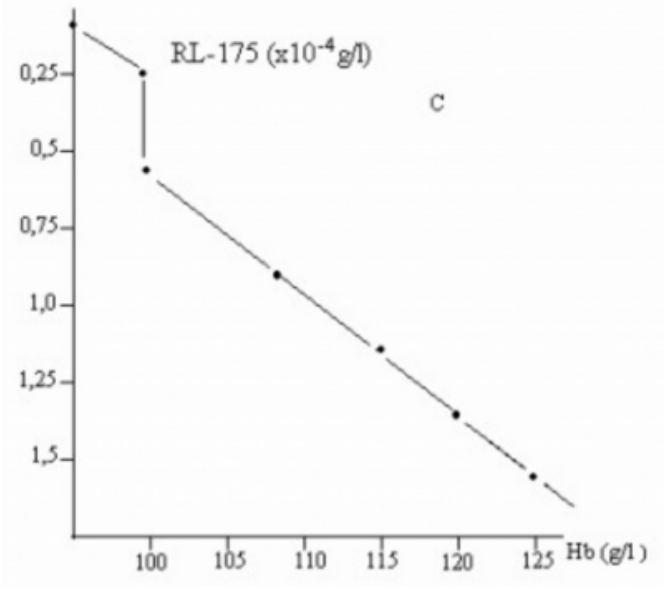
Preparation RL-175 in the dose 100 mkg/ml results in partial inhibiting the influence on the inclusion speed of [^{3} H] – thymidine. Synthesis of DNA increases in doses 10 and 1,0 mkg/ml of the pointed preparation. In its turn, RL-S practically wholly inhibits synthesis DNA and simultaneously considerably stimulates synthesis of RNA: inclusion [^{3} H] – uridine increases 214 % at doses of 10 mkg/ml regarding on the control index.

Preparations RL-175 and RL-S exert considerable influence on the correction of normal human and animal hemopoiesis (Table 1). There was revealed concentrational dependence of production of hemoglobin (Hb), erythrocytes (Ery) and leukosytes (Leuk) in the peripheral animal flood from dose RL-S. Optimal are: for the female rats the summary dose is 2,4x10 $^{-5}$ g/l and for the hemoblasted cows – 15,9x10 $^{-5}$ g/l.

Hb and Ery in experimental cows increased regarding initial indices on 30-53 % and in healthy rats these indices reached 67 % and 140 % correspondingly (Table 1). By this effect of the preparation RL-S action after experiments on Hb, Ery and Leuk indices practically changes slightly during 180 days (the period of observations). By serologic diagnostics revealed after a treatment course by preparations RL-S the displacing of antibodies contents agglutination in 1 ml serum of blood of cows with brucellosis from 200-400 IU/L (International Units on a litre) up to zero (agglutination in all doses of serum was absent).

On Fig. 1c there are presented average results of anemia correction in clinics by the preparation RL-175 in five patients with acute lymphoblosted leucosis (ALL) and blasted crises. Patients with chronic kidneys insufficiency didn t get into remission. With the introducing RL-175 in a dose of 1,5x10 $^{-4}$ g/l in patients with ALL stable correction of anemia (up to the norm associated with the malignant tumor) was observed.

Figure 5



It should be marked that the difference from RL-S preparation and RL-175 does not considerably influence indices production of red blood. However, with the account that RL-S did not pass clinical approbation the decision was made to use RL-175 which had undergone preclinical trials. The preparation RL-175 was introduced per orally in patients with ALL.

DISCUSSION

Excluding outstanding bioenergetic function of ATP (moving, active transport, biosynthetic metabolism) one more amplification (strengthening) of impulse is known [^{5}]. As response to the action of some polypeptide hormones, risk factors, mitogenes and cytokines (endogenic irritators) on plasmatic membrane of the animal cell in anaerobic conditions ATP from ADP and Pi is synthesized.

The displacing of thermodynamic standard balance ADP + Pi ATP in 10 ^{16} times regarding physiologic norm by the preparations RL-175 and RL-S is associated by considerable synthesis of ATP on tumorous cells plasmatic membrane against its zero concentration in neoplasia [$_{6}$]. The energetic currency forming on a plasmatic membrane of a tumourous cells becomes again accessible to the endogenic irritators, particularly to the insulin having receptor sites to ATP [^{6},$_{7}$]. Thus, it provides the initiation of the mechanism of receptors and non receptors tirozinekinase for the cells growth factors

and conduction of further proliferative stimulus into the nucleus.

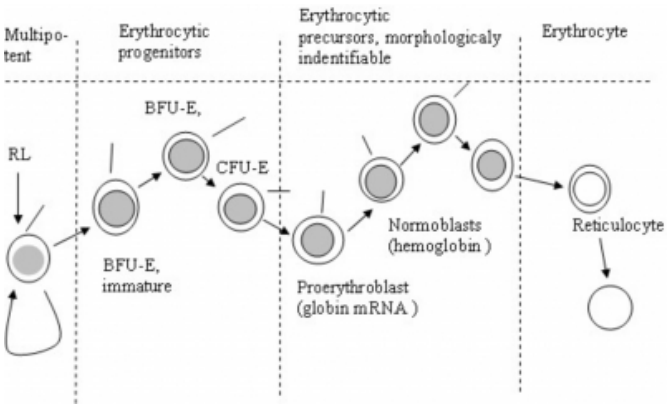
By the confirmation that investigated preparations RL series are a universal stimulus transducting ATP transmitters as a booster to the cell growth and cellular proliferation RL can control regulation of DNA and RNA synthesis in tumourous cells. The development of an excessive pool of RNA in $CaO_{\square}$ cells [$_{8}$] is connected with ‘‘editing'‘ lengthening of matrix RNA chain by the reverse translation mechanism increasing the DNA program according to the scheme:

general pool RNA DNA synthesis of protein.

Pathogenesis of the anemia manifests in the immune system function especially in its macrophagal link [$_{9}$]. Considerable strengthening of developing and maturing of erythrocytes in anemia correction in patients suffering chronic kidney insufficiency (Table 1 and Fig, 1) confirmed the RL influence on the induction of endogenic erythropoietin (EPO) in kidneys with parallel increasing of its sensitivity in bone marrow (Fig.2).

Figure 6

Fig. 2 The major differentiation and maturation steps in erythropoiese (CFU-GEMM: colony-forming unit granulocyte, erythrocyte, macrophage, megakaryocytic, BFU-E: burst-forming unit-erythroid; CFU-E: colony-forming unit-erythroid). Modified from [9].



Moreover similar considerable influence of preparations (more than on 25%) testifies to a high reliability as of maturing induction of erythroid cells – precursors (BFU-E; CFU-E), so of a correction of blast cells reverse entering into the normative proliferation cycle. After a course of RL-175 treatment of the patients with ALL the contents of blast cells in the myelogram has been decreasing in average from 70 to 3,0%. Earlier,we discovered the high sensitivity of endocrine glands to the investigated RL preparations.
Systemic analysis of the contents in the flood of animals (rats, cows) such as sexual hormones (testosteron, estradiol, progesterone), glucocorticoid hormones (corticosteron, 11-desoxycortisol, cortisol), luteinazing hormone (LH) uncovered considerable RL influence on key regulative mechanisms in the system of direct and indirect relations of hypothalamus – hypophysis – endocrine glands.

Thus, RL preparations are able to correct anemia through amplification of intracellular signaling apparently by tyrosine protein phosphotage, induction of cellular gene transcription including hemopoietic growth factors as well as maturing of erythroid cells (forerunners BFU-E, CFU-E) and considerably overcome human urinary EPO and recombinant human rHu-EPO [^{9}]. In all likelihood, rHu-EPO is the best selling drug in the world (estimated sales 5,000 millions US $ per year).

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