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

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Citation

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 \(\rightarrow\) hypophisis \(\rightarrow\) gonades \(\rightarrow\) 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).

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).

<table>
<thead>
<tr>
<th>Animal, the physiological state</th>
<th>Dose of RL-S g/l</th>
<th>A. number of injections</th>
<th>Total dose g/l</th>
<th>Hb</th>
<th>Ery</th>
<th>Leuk</th>
<th>% of increasing</th>
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<tr>
<td>Healthy cows ((n=30))</td>
<td>0.0</td>
<td>5</td>
<td>2.4</td>
<td>0.486,0</td>
<td>134,01</td>
<td>7,541,3</td>
<td>50,805,1</td>
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<td>Controlled ((n=10))</td>
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* 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 zootecnich and veterinarian 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 0.8 x 10^{-5} \text{g/l} and cows were also dosed 3 times at different
dose levels of RL-S (optimal dose 15.9 x 10^{-5} \text{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 leukemia 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 (1.14 x 10^{-5} \text{ mole•c against} 
0.67 x 10^{-5} \text{ molec in control}) by 60-80 \% (P 0.01)\[4\].
Qualitative thin-layer and gas-fluid chromatography
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 (\(\Delta G\)) 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. \(\Delta G\) discharged at hydrolysis
of energetic value in intact erythrocytes, muscle and rat s
liver \[5\]:

**Figure 2**

\[
\Delta G = \Delta G^{0} + 2,303RT \log \frac{[ADP][P_i]}{[ATP]} = -51,9kJ/mole,
\]

*where \(\Delta G^{0}\) – standard free energy, \(R\) – gas constant;
\(T\) – absolute temperature and \(P_i\) – 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 \[^1\], than difference in - 21.4kJ/mol comprises
the standard energy (\(\Delta G^{0}\)) of displacing the preparations RL
of mentioned above balanced system (pKa) in the pool of
multifermential complex of respiratory chain in 10^{-10} times
regarding of balanced (pK a) uninvolved spontaneous
passing of molecule A into B pK_a = - 1.15 x 10^{-3} \[^1\].

**Figure 3**

\[
pK_a = \frac{[B] \cdot [ATP]}{[A] \cdot [ADP] \cdot [P_i]} = 0.28 \times 10^{-7}
\]

*where for A spontaneously turning into B by \(\Delta G^{0} = -21.4b\)
KJ/mole pK_a = 5.62 x 10^{-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 \[^1\].

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\(_\gamma\) 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.
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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).


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 $2,4\times10^{-5}$ g/l and for the hemoblasted cows – $15,9\times10^{-5}$ 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 lymphoblasted leucosis (ALL) and blasted crises. Patients with chronic kidneys insufficiency didn’t get into remission. With the introducing RL-175 in a dose of $1,5\times10^{-5}$ 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 $ADP + Pi \rightleftharpoons ATP$ 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 [5, 6].

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 transducing ATP transmitters as a booster to the cell growth and cellular proliferation RL can control regulation of DNA and RNA synthesis in tumorous cells. The development of an excessive pool of RNA in CaOγ cells 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. 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 (testosterone, estradiol, progesterone), glucocorticoid hormones (corticosteron, 11-desoxy cortisol, 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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**References**
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