NEW MECHANISM OF THE WATSON-CRICK MODEL OF GENETIC INFORMATION TRANSMISSION BY EXAMPLE OF NON-CYTOKINE REGULATION OF HEMOPOIESIS

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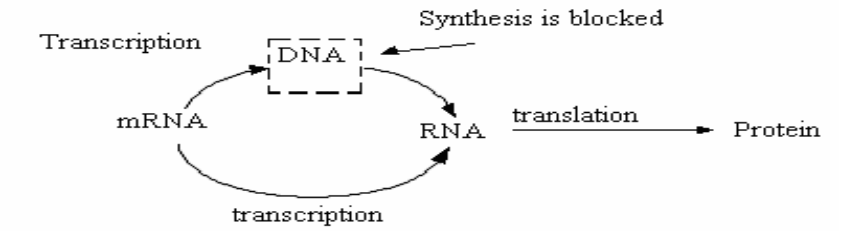
Our experiment proves conception, that interference of radiation frequency of wave energy hγ≈10^{13} Hz of ATP synthesis in plasmatic part of respiratory chain and resonance absorption of this energy frequencies by cellular genetic elements is physical nature of initiating signals to cell growth and cell proliferation through IAK-STAT-Kinase. As a result, it leads to smoothing frequencies of many conformational enzyme-substrate waves, and transmission of molecular signal from plasma membrane inside the nucleus according to the law of Determination L with energy of activation barrier Eact ≈ 0. It is shown, that influence of N-substituted 3-oxipyredine salts on normal and tumor cells results in considerable activation of RNA-polymerases with corresponding amplified duplication of normal RNA-transcripts and reduplication of newly created genetic information as a result of post-transcripts splicing into RNA-dependant DNA-polymerases. Therapeutic effects of the experimental compounds could substitute in clinic rHu G-CSF, rHu EPO, rHu TPO and other known remedies for treatment of anemia and thrombocytopenia of different genesis.

Realization of genetic information contained within the structural model of duplex DNA by J. Watson and F. Crick (1953) through transcription and translation is regarded as a universal code for all living organisms [1, 2]. Hereditary information is transmitted from DNA-template to protein synthesis in the following direction:

$$\text{DNA} \xrightarrow{\text{transcription}} \text{RNA} \xrightarrow{\text{translation}} \text{Protein}$$

However, we have presented experimental evidences in works [3-8], which overturn this common theory of molecular genetics. In particular, when DNA synthesis is almost completely inhibited as a result of the influence of N-substituted 3-oxipyredine salts on tumor cells of human ovarian carcinoma (CaOγ) at the same time RNA synthesis is stimulated by 214% compared with control values. At subcellular level (mitochondrion), cellular level (yeast, microorganisms, malignant cells) and organism levels (100000 drosophilas, 1200 non-pedigree rats and Vister rats, 10000 chickens, 30 calves, 52 young pigs, 107 cows with hemoblastosis and 36 monkeys) we showed, that the experimental salts cause epigenetic (non-genetic) selective stimulation of normal metabolic and physiological processes at higher animals [9].

The obtained experimental results could only be explained from position, that mRNA is a template of protein synthesis at higher animals in direction of selective distribution of normal copies of RNA-transcripts from one generation of animals to another during embryogenesis:



In 2005 epigenetic inheritance of mutant trait was proved for higher plants [10], and in 2006 – for higher animals [11]. Results provided in the latest works sparked discussions among specialists. In particular, Paul Soloway from the Cornell University marked in the covering letter to work by Minoo Rassoulzadegan and co-authors [11], that this research challenges modern ideas about hereditary.

But, to our opinion, there's no experimental ground not to regard DNA as a common carrier of hereditary information. Inheritance of mutant gene Kit at animal generation with normal genotype Kit+/+ on condition of different genetic variations of crossing [11] don't prove, that genetics laws are not working. They show that under influence of new experimental data new aspects of half-conservative mechanism of DNA reduplication are being revealed.

This report is concerned with experimental basing of a new mechanism of genetic information transmission in the Watson-Crick reduplication model on example of non-cytokine regulation of hemopoiesis of normal and tumor cells at higher animals and men by N-substituted 3-oxipyridine salts.

Activation of cell genome by resonance absorption of energy frequencies radiated by plasmatic ATP

Cells as a main structural functional unit of all living organisms normally adequately changes in response to external and internal signals towards activation of growth factors and conduction of proliferative stimulus into the cell nucleus [12].

Signal molecules include polypeptide hormones, growth factors, mitogenes, cytokines and some other molecular structures. Interaction of these molecules with specific receptors on cell membrane is an extremely difficult and to a large extent a poorly studied process.

To date, the number of basic interleukins (IL) has reached 23. Cytokines also include interferons, factors of tumor necrosis, growth factors, chemotoxic factors and others.

Effect of such big number of cytokines in regulation of hematogenesis and immunogenesis is mediated by specific receptors on cellular plasma membrane, for example, for family IL-6 – it's gp 130 [13]. Intermolecular reception and signal transduction from membrane to cell cytoplasm causes dimerization of gp 130. In the framework of molecular ideas it is a starting mechanism for activation of transducer chain, associated by attraction of receptor and non-receptor «family» of IAK-kinases.

The main purpose of A-Iak (Iak 1; Iak 2; Iak 3 and Tyk 2), Src-family (Blk; Fgr; Fyn; Lck; Hck; Lyn), C.Fps/Fes-family (c-fes type), D.Tec/Btk-family, E.Syk/ZAP70-family, VRK3, cAbl and a great number of other intracellular tyrosine kinases is phosphorylation and dephosphorylation of tyrosine bases of cytokine receptors' signal chain aimed at creation of specific areas for attaching SH2 – domains [13, 14]. It should be mentioned, that today only KINEOS antibody microarray service provides ultra sensitive analysis of about 600 signal-transducing proteins in phosphorylised condition.

Tyrosine bases of intracellular receptor, activated by phosphorylation, attract 7 inactive forms of Stats (signal transductors and activators of transcriptions), containing SH2 – domains. Tyrosine kinases activate Stats be means of phosphorylation, causing their dimerization, then they move away from the receptor and penetrate into cell nucleus, causing activation of specific DNA elements (fig.1).

So, this simple scheme of cytokine interaction with cells shows, that cytokine effects on cell come as a result of multiversion interactions, and conduction of cytokine signals to genetic elements is a very difficult and multi-step process.

Simple calculations show, that even having minimum 3-variant attempt and only 50 negative and positive regulators of signal transduction of cytokine stimuli inside the cell and ability of this cell to express specific properties at genetic level at a speed of $10^{10}s^{-1}$, that would require 3^{50} or 10^{50lg3} x 10^{-10}s 0 about 10^{7} years!

So, our calculations prove, that classic interpretation in the framework of molecular theories, for example, homodimer gp130 = gp130, as it were starting mechanism in initiation of signal from cytoplasmatic part of

gp130 to cell nucleus through JAK-STAT-KINASE [13] – is just nonsense.

To understand the nature of transcriptional genes activation by signal molecules we studied how synthesized salts [4, 6] of N-phenyl-3-oxipyridine (RL-175), N-hydroxyphenyl-3-oxipyridine (RL-S) and N-tolyl-3- oxipyridine (RL-3) influence on succinate oxidation rate in mitochondria of rat liver. It was shown, that under the influence of the experimental salts succinate is oxidized 60-80% stronger than in control: $1{,}14x10^{-6}$ s^{-1} compared with $0{,}67x10^{-6}$ s^{-1} in control, that corresponds with the same size acceleration of ADP phosphorylation into ATP. In skeletal and cardiac muscles of test rats, fed by experimental compounds, synthesis of ATP in vivo also increases by 2 and more times.

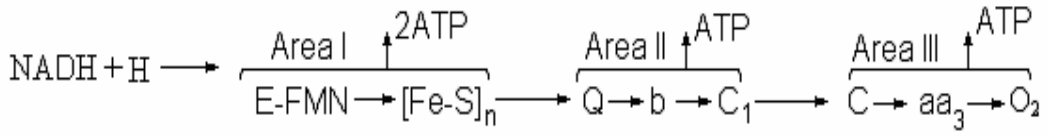
Authentic free energy (ΔG) of ATP synthesis in the above mentioned muscle cells when concentration of ATP, ADP and Pi are correspondingly 40; 0,93 and 8,05 мМ, pH value - 7,0 and provided 25^0C, equals as follows:

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

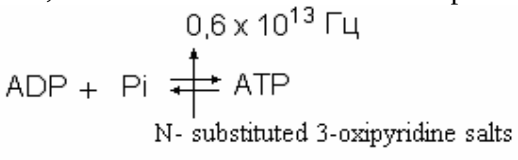
Where ΔG^{01} – standard free energy; R – gas constant; T - absolute temperature and Pi – phosphoric acid.

In standard thermodynamic conditions from -51,9 kJ/mole 30,5 kJ/mole are required for synthesis of one molecule ATP from ADP and Pi. The residual of -21,4 kJ/mole is standard energy (ΔG^{01}) of radiation in pool of multi-enzymatic complex of respiratory chain, which equals $Keq = 10^{-21{,}4\ /\ 5{,}7} = 5{,}62x10^3$ Hz.

Apparently, under the influence of N-substituted 3-oxipyridine salts 4 ATP molecules are synthesized in 3 key parts of respiratory chain. Normally there are 3 molecules [15].



Taking this into account, frequency of radiant energy during synthesis of 4 ATP molecules in three parts of respiratory chain is $0{,}56x10^{13}$ Hz and this value is comparable



with the frequency of resonance energy absorption by mitochondria of human hepatic cells ($3{,}18x10^{13}$ Hz) [16, 17].

It should be mentioned, that standard energy (ΔG^{01}) of electron flow from NADH to O_2 in the above mentioned respiratory chain equals [15, 18]

$$NADH + \tfrac{1}{2} O_2 + H^+ \rightleftharpoons H_2O + NAD^+$$

$\Delta G^{01} = -220{,}08$ kJ/mole.

122 kJ/moles are used for synthesis of 4 ATP molecules. Then from Planck equation $E=h\gamma$ of proportionality between absorbed and radiated energy at the same frequency in single act we can conclude the following: about 77,0 kJ/mole ($3{,}18x10^{13}$ Hz) from the remaining 91,5 kJ/mole fall at energy radiated by mitochondria of human hepatic cell, and the rest energy – heat dispersion. Radiation frequency calculated for mitochondria $0{,}6x10^{13}$ Hz - $3{,}18x10^{13}$ Hz is also comparable with frequencies of resonance energy absorbed by nuclear cell structures [16, 17]: with body cell of mammal ($2{,}39x10^{12}$ Hz), nucleus of body cell of mammal ($9{,}55x10^{12}$ Гц), human cell genome ($2{,}5x10^{13}$ Hz), chromosomal interphase ($7{,}5x10^{11}$ Hz) and metaphase ($1{,}5x10^{13}$ Hz), with genome subunits, which code protein with molecular weight of 50 thousand dalton ($< 9{,}7x10^{14}$ Hz) and other genetic elements.

For reference let us note that molecular weight of the known hemopoietins varies within the limits of 15-50 kDa (glycoprotein-IL-6 has 25 kDa) [19]. The level of molecular weights G-CSF, EPO and TPO is considerably smaller than 50 kDa [20].

So, from Planck equation $E=h\gamma$, which says that amount of radiated and absorbed energy in a single act of system is proportionate to frequency (γ), we can conclude, that physical nature of initiation of signal from cytoplasma to nuclear cell subelements lies in interference of frequencies radiated by plasmatic ATP and resonance absorption of this energy frequencies, for example, by genes of eukaryotic RNA-polymerases.

Interaction of equal frequencies of energy radiation and absorption energy leads to smoothing of quantum frequencies of conformational enzyme-substrate waves which results in activation or inhibition of molecular mechanisms of cell growth induction, amplification and transduction of this signal stimulus from plasma membrane to myelo-proliferative genes and vice versa, with energy of activation barrier $\Delta Eact \approx 0$, according to the law of Determination, during chemical transformations (fig.1) .

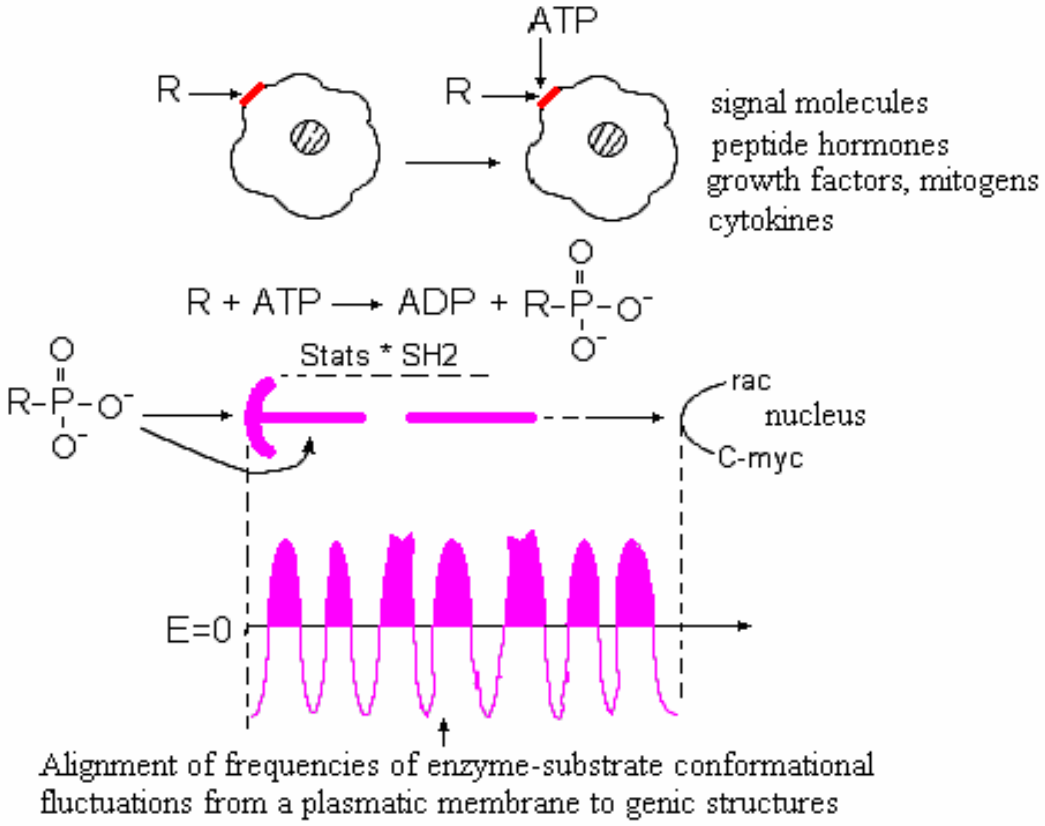


Fig.1. Scheme of intracellular conformational oscillatory–resonant signal transmission to transcriptional genes according to Law of Determination (L) [21]. Difference of free energy (E) between closed, spontaneously reacting initial and final systems $E = \Delta H + RT$ (where ΔH – enthalpy; R – gas constant; T – absolute temperature) – is an energy of activation barrier. Expression E is associated with the principle of uncertain coordinate and impulse (speed) of reacting particles in a well-konwn Geizenberg ratio, from which it follows, that not every collision leads to reaction product. For example, each active enzyme-substract collision accounts for 200 encounters [12]. In chemical transformations where $E \approx 0$ each encounter of particles is effective. These processes run according to the principle of Determination [21] unlike reactions where $E \gg 0$ and reacting particles need to acquire this energy in order to enter a chemical reaction during collision.

A number of authors have to some extent similar ideas as in our research in questions of synchronization of waves of many endogenous oscillators depending on external exogenous or endogenous factors [13, 16, 17].

Non-cytokine regulation of normal and tumor hemopoietic cells (G-CSF, EPO and TPO)

Cytokines G-CSF (Granulocyte colony-stimulating), EPO (Erythropoietin) and TPO (Thrombopoietin) don't have receptor tyrosine-kinas activity but have common chains in intracellular part of receptors [13, 14].

Extracellular receptor part of these chains is responsible for specific binding, and intracellular – for formation and transmission of cytokine signals to transcriptional genes. In response to influence of cytokines G-CSF, EPO and TPO on extracellular hemopoietic specific receptors of animal or human cell in aerobic conditions ATP is synthesized from ADP and Pi (fig.1) unlike its zero concentration in tumor cells [22].

Following these classic molecular theories of hemopoiesis regulation, we should have expected extremely low sensitivity of normal and tumor hemopoietic cells to the studied N-substituted 3-oxipyridine salts. This conclusion was based on the following: firstly, in experiment we showed [3], that N-hydroxyphenyl-3-oxipyridine almost completely inhibits activation of DNA in tumor cells of human ovarian carcinoma (fig. 2a, b) and secondly, N-substituted 3-oxipyridine salts do not inhibit tyrosine kinases, on the contrary, they considerably induce activation of receptor and non-receptor tyrosine kinases for growth factors of cells.

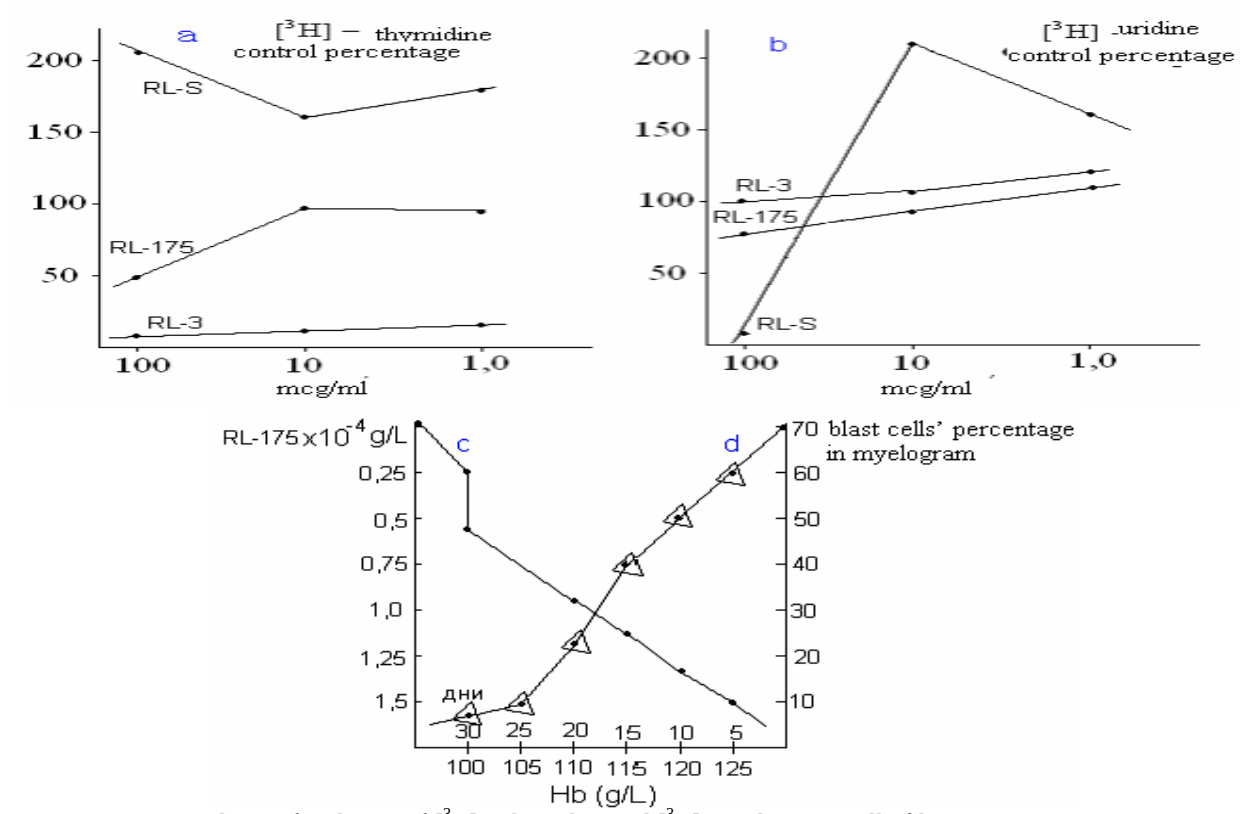


Fig. 2. Dependance of inclusion of [^{3}H] – thymidine and [^{3}H] - uridine in a cell of human ovarian carcinoma on concentration of preparations RL (a, b). Hb concentration icreases at 5 patients with ALL with chronic renal failure (c) and blast cells content in myelogramme reduces depending on the dose of RL-175. Diagnosis of these patients was classified in Russia's leading hematologic centres. All research on marrow cells, diagnostic punction before and after treatment by preparation RL-175, were conducted in the same scientific centres.

Due to this reason the experimental salts should have activated mechanisms of intracellular transmission, first of all, of tumorigenic signal stimulus to transcriptional genes [13, 22].

Results

The experimental compounds considerably induce non-cytokine growth regulation, differentiation and correction of normal and tumor hemopoietic cells (table 1-3, fig.2 c, d). We revealed how hemoglobin (Hb), erythrocytes (Ery) and leukocytes (Leuk) production in peripheral blood of animals depends on the dose of N- hydroxyphenyl-3-oxipyridine salt (RL-S). Optimal doses are: for female rats – 3-time total dose 2,4 $x10^{-5}$ g/L or for hemoblastosis cows – 15,9 $x10^{-5}$ g/L.

Table 1 Content change of haemoglobin (Hb), erythrocytes (Ery) and leukocytes (Leuk) in peripheral blood of animals depending on injection of different doses of RL-S ($p< 0,05$)*

Animal, physiological condition	Dose RL-S, g/L per heads $x10^{-5}$	Number of injections	Total dose g/L $x10^{-5}$	Hb /L	Ery $x10^{12}$ /L	Leuk $x10^{9}$ /L	Increase rate	
							Hb	Ery
Healthy rats (n=20):								
Control (n=10)	-	-	-	104±6,0	3,16±0,2	10,0±0,1	0,0	0,0
Test (n=10)	0,8	3	2,4	174±1,0	7,5±0,5	6,0±0,2	67,3	137,4
Healthy cows (n=50):								
Control (n=10)	-	-	-	98±6,0	6,4±0,5	6,9±0,5	0,0	0,0
Test (in each series n=10)	2,8	3	8,4	114±10,0	4,0±0,4	6,9±0,4		
	2,8	6	16,8	126±9,0	9,6±1,5	9,1±1,3		
	5,3	3	15,9	129±7,0	9,8±1,0	6,6±1,8	31,6	53,1
	5,3	6	31,8	89±16,0	7,5±1,8	10,0±1,3		
Cows with brucellosis (n=16):								
Control (n=6)	-	-	-	102±9,4	6,8±0,6	7,4±1,5	0,0	0,0
Test (n=10)	5,3	3	15,9	156±10,2	9,8±0,6	8,3±1,3	53,0	44,0
Cows with hematoblastosis:								
Control (n=10)	-	-	-	98±6,6	6,9±0,5	21,0±3,1	0,0	0,0
Test (n=10)	5,3	3	15,9	142±8,0	10,2±1,0	6,7±0,8	45,0	48,0

*Preparation was injected to animals intravenously during 7 days.

Hb and Ery content at healthy test cows increases from initial values by 30 and 53 % respectively, at rats - 67% and 140% (table 1); at hemoblastosis animals – 45,0 and 48% respectively. The effect of N-hydroxyphenyl-3-oxipyridine on Hb, Ery, Leyk values and other blood elements practically remains during 180 days after tests (observation time).

The same values for cows with brucellosis have increased: Hb - by 53% and Ery - 44%. Serum diagnostics revealed that after the course of treatment agglutination titer of antibody content in 1 ml of blood serum of sick animals shifted from 200-400 IU/L (International Units Liter) to zero (there was no agglutination in all serum doses).

Fig. 2c and 2d show average results of anemia correction in clinic during tests of N-phenyl-3-oxipyridine salt on 5 patients with acute lymphoblastic leukemia (ALL) c blast crisis. Patients with chronic kidney disease did not remit after traditional course of therapy using human recombinant rHu-EPO. At the same time after the course of treatment using

the experimental preparation in total dose 1,5 x10^{-4} g/L the same patients (ALL) had stable correction of anemia to norm and reduced content of blast cells in myelogram from 70% to 3,0%.

Tables 2 and 3 show results of clinical hematological research into effect of preparation N-phenyl-3-oxipyridine (RL-175) on 15 patients aged from 6 to 65 (average age is 26), with thrombocytopenia of different genesis (central and peripheral). Before approbation by the studied preparation all patients underwent complete courses of therapy in Russia's leading Hematological Centers in 1990-2000.

At the 3rd and further stages of relapse effect of traditional treatment at all patients reduced almost till zero level (positive anti-tumor effect). Patients did not remit, they had an unfavorable prognosis for the disease.

Depending on the clinical peculiarities of disease course all 15 patients were divided into 3 groups. The 1st group included a 16-year old boy diagnosed with chronic immune thrombocytopenic purpura (ITP).

The 2nd group included 5 female patients with hereditary idiopathic thrombocytopenic purpura, and the 3rd – 9 female patients with thrombocytopenia, caused by aplasia of myeloid stem cells.

Criteria for choosing these 20 patients for approbation by N-phenyl-3- oxipyridine (RL-175) were the following: voluntary agreement of patient, strict verification of diagnosis, evaluation of patient's condition, very low results of traditional therapy and others, according to ethic norms and requirements, and also standards, stated in Helsinki Declaration of 1975 (resisted in 1983).

Patient G.V. - a 16-year-old boy - applied in December, 1991 to the North-Ossetian center of medical-biologic problems (Vladikavkaz, Russia) diagnosed with immune thrombocytopenic purpura (ITP). Since June, 1991 he has undergone treatment in several Russian clinics, but despite higher dose of prednisone in combination with Immune Gammaglobuline (IVIG), as well as transfusion of thrombocytes concentration he never remitted.

Acute condition was accompanied by nose and gingival bleeding, hemorrhagic skin rash and weakness.

On 01.21.1992 patient G.V. began to take preparation N-phenyl-3-oxipyridine per os. By 02.16.1992 complete remission has occurred according to clinical hematological results: Thrombocyte (Thro) - 190x10^9 g/L; Hemoglobin (Hb) -150 g/L; Erythrocyte (Ery) - 4,6x10^{12} g/L; Color index -1,0; Leukocyte (Leyk) - 8,0x10^9 g/L; Eosmophil (Eos) – 2% Rod Like nuclei (rode. nuc) – 4%; Segmented nucler (segm) – 66%; Lymphocyte (Lymph) – 18% и Monocyte (Mono) – 6%.

2 months later (03.19.1992) the number of thrombocytes reached Thro – 235x10^9 g/L with complete normalization of blood hemapoiesis being observed: Hb - 122 g/L; Ery – 4,2x10^{12} g/L; Leyk – 6,6x10^9 g/L; Baso – 7%; rode. nuc – 3%; segm – 66%; Lymph – 18% and Mono – 6%.

Table 2 Average number of platelets at patient with chronic immune thrombocytopenic purpura (ITP).*(P< 0,05)

Year	Treatment	Mean plt x 10^9/L
June 1991	Prednisone	52
08.20-10.15.1991	Prednisone, danazol, vinblastine, IVIG**	42-52
10.29.1991	Prednisone	220
11.19-12.10.1991	Prednisone, IVIG	42-52
01.21.1992-13.19.1992	***RL-175 (170 mg/m^2)	190 235
04.19.1992-01.01.1996	***RL-175 (340 mg/m^2)	220 220

*In work [23] an example is provided of successful treatment of a patient with ITP by monoclonal antibody Retuxan (rituximab). Woman, aged 35, during 10 years underwent traditional treatment (mean plt 42-15 $x10^9$ g/L) which was unsuccessful (prednisone, cyclophosphamide, vinblastine, IVIG**, vincristine, cyclophosphorine, colchicne, azathioprine). But after two injections of rituximab in dose of 375 mg/m^2 the patient remitted (170$x10^9$ g/L). Duration of the preparation's effect is not mentioned.

**IVIG – Immune Gammaglobulin.

***RL-175 – N-phenyl-3-oxipyridine salt.

From January, 1992 to January, 1996 patient G.V. received preparation RL-175 in total dose 0,34 g/m^2 (table 2). In this period according to clinical hematological results an his objective condition the patient experienced complete and stable remission.

Table 3 presents results of how preparation RL-175 induced maturation of thrombocytes at 5 patients (n=2-6) with hereditary ideopathic TP and 9 patients with disturbed production of thrombocytes in bone marrow (n= 7-15). All 14 patients during 20 days received preparation RL-175 in total dose 150-170 mg/m^2. Frequency of objective (full or partial regress) answers was 70-90% with median duration of effect - 6 - 8 months. Effectiveness of the experimental preparation at further therapy stages was higher than 50%.

Table 3 Induction by N-phenyl-3-oxipyridine of thrombocytes (Thro) and erythrocytes (Ery) production at patients with thrombocytopenia (TP) of central and peripheral genesis (p<0,05)

Factors of TP development (n – number of patients)	**F-sex Age**	**Mean Thro x 10^9/L**		**Mean Ery x 10^9/L**	
		Before treatment	**After treatment**	**Before treatment**	**After treatment**
Idiopathic thrombocytopenic purpura:					
n=2	6	60	190	4,1	4,5
3	16	42	320	4,1	4,1
4	19	16	135	2,8	3,3
5	19	16	150	2,8	3,5
6	6	8	220	1,6	3,1
Hematosis aplasia:					
n=7	17	42	310	4,0	4,3
8	65	17	220	4,4	4,5
9	57	15	151	4,1	4,1
10	41	247	570	3,8	5,0
11	61	190	230	3,4	4,1
12	13	170	225	4,4	4,5
13	20	60	420	2,3	4,0
14	15	25	270	2,2	3,8
15	17	19	245	2,0	3,5

Clinical hematological results on TP correction of marrowy and peripheral genesis (table 2 and 3) show, that the experimental preparation has a stronger effect on proliferation and differentiation of megakaryocytic bursts (BFU-MK) than the known remedies, including Rituximab [23]. Apparently, fast growing number of hematoblasts in peripheral blood (in 6-9 times at patients n=2-9, 13-5, table 3) during 10-20 days under influence of very small doses of N-phenyl-3-oxipyridine prove high reliability of induction of endogenous TPO production. Another evidence is that in our experiment there were no abnormal sequestration (table 3 n=10-12) as a result of different time terms of cytokine receptors expression by the experimental preparation on maturation and cell differentiation of hemapoietic cells: there is data about receptors to TPO in early pluripotent stem cells, as well as late, receptors to EPO form at later maturation stages of these cells. Otherwise there would be an abnormal sequestration – speed of destruction and abnormal distribution of thrombocytes and erythrocytes in the same channel of reticuloendothelial system.

Discussion

Erythropenia and thrombocytopenia are caused by aplasia of myeloid stem cells. Their differentiation and maturation goes in reticuloendothelial system (RES). High reliability – an increase of more than 25% comparing with control values, induction of simultaneous maturation in RES of both erythroid precursor cells (BFU-E, CFU-E), and myeloid stem cells till megacarciocite with the following fast production of erythrocytes and thrombocytes (table 1-3, fig. 3) prove that activity regulation of endogenous G-CSF, EPO and TPO N–substituted 3-oxipyridine salts is being corrected. These hemopoietins are one of the most important regulators of erythropenia and thrombocytopenia [20, 23].

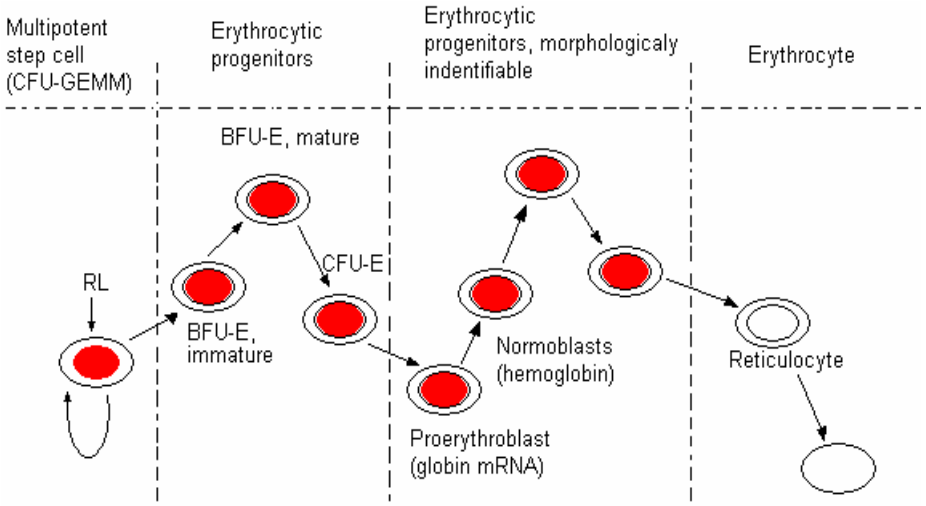


Fig.3. The major differentiation and maturation steps in erythropoiese (CFU-GEMM: colony-forming unit granulocy hrocyti, macrophage, megakaryocytic, BFU-E: burst-forming unit-erythroid; CFU-E: colony-forming unit-erythroid) [8].

The above provided experimental and clinical results are difficult to explain using classic molecular theories. First of all, it was not clear how N-hydroxyphenyl-3-oxipyridine salt activates proliferation and differentiation of normal and tumor hemapoietic cells, as it almost completely blocks DNA activity in tumor cells and at the same time inhibits RNA synthesis in these cells by 214% (fig. 2).

On the other hand, the experimental compounds considerably influence on synthesis of plasmatic ATP in normal and tumor cells, that is accompanied by activation of tyrosine kinases and growth factors, which have receptor sites to this energy currency. In this case under influence of N-substituted 3-oxipyridine mechanisms of intracellular transmission of tumorigenic signal stimulus inside the cell

nucleus should be activated in thr first turn, which our experiments do not prove [3-9, 21].

We may assume, that activation of transcriptional genes of pluripotent and then myeloid stem cells, as well as tumor cells' reverse entering into the normal cycle of cell proliferation prove an idea, that not DNA [3, 10, 11], but RNA is carrier of hereditary information. But despite looking obvious, this theory does not allow to understand how living organisms evolutionally accumulated genetic information and realise it during ontogenesis. Similar to transduction of cytokine or other signal stimuli on comlex and multivariant cascade of receptor intracellular chain, as it would require according to our calculations astronomical time of 10^6~10^7 years!

The above shown contradictions of experiment and theory could be easily overcomed if we accept a new mechanism of Watson-Crick model of genetic information transmission (fig.4).

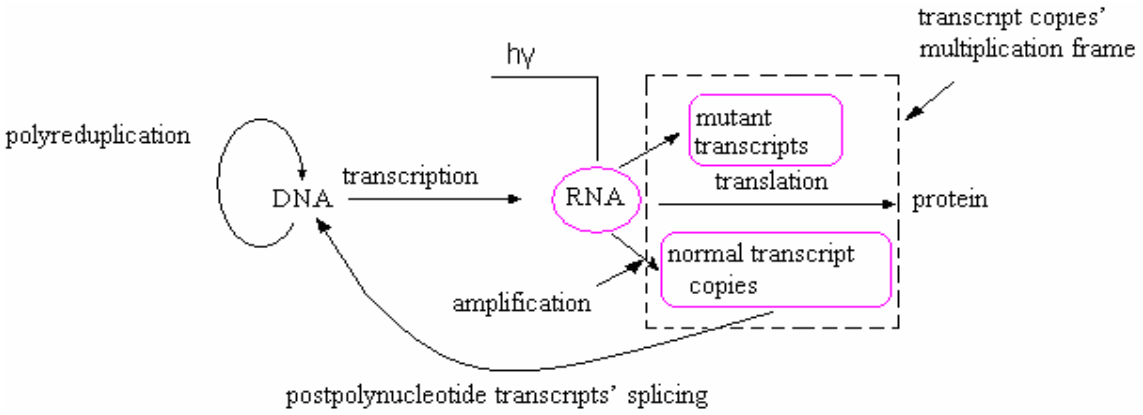


Fig. 4. New interpretation of central belief of molecular genetics, which takes into account a possibility of «edited» changes to post-transcripts and creation of new genetic information on RNA-dependant DNA-polymerases (polyreduplication). Frequency of mistakes among newly created RNA-polymerases of polynucleotide chain is 10^5 times higher, than during DNA synthesis. Reliability of RNA synthesis is achieved by numeroues copies of RNA-transcripts being made from one gene[2].

Equal levels of radiant frequancy of plasmatic ATP energy $h\gamma \approx 10^{13}$ Hz and resonant absorption of this energy frequencies ($h\gamma \approx 10^{13}$ Hz) by structural subunits of RNA-polymerases, and adequate amplifying of multiplication of normal RNA-transcripts copies and polyreduplication of newly created genetic information as a reslut of splicing of post transcripts into RNA-dependant DNA-polymerases, form a base of a new interpretation of molecular genetics' central theory.

Experimental and clinical results shown above, as well as results published in works [3-9] are reliable evidence in support of the new theory of molecular genetics. It also let explain abnormalities in inheritance of mutant trait with normal genotype at higher plants [10] and animals [11], which occur as a result of molecular mistakes during different intracellular genetic variations [2].

Experimental and clinical procedures

In experiment we used 86 cows of different age (4-8 years) and breed. Among them, according to the Veterinary Service, 20 animals were ill with hematoblastosis and 16 animals – brucellosis. They were divided into 9 groups of 10 animals (n=10) each. Healthy and sick cows divided into groups according to breed, age and level of morbidity. The animals lived in usual zootechnical and veterinary conditions of life and feeding typical in the republc of North Ossetia-Alania (Russia). Tests and observations with the cows were conducted during 7 months (term of observation). After tests animals underwent clinical examination weekly.

We also used 2 groups (control and test) of 10 healthy female Vister rats with mass of 120-150 g.

Preparation N-hydroxyphenyl-3-oxipyridine (RL-S) was injected to animals

intravenously using 0,9% isotonic solution: to rats - 3 times, $0,8x10^{-5}$ g/L and to cows also 3 times in different doses of the preparation (optimal dose $15,9x10^{-5}$ g/L) during 7 days (each alternate day).

Hematological test was the main criterion to choose optimal dose of the preparation. Results of clinical hematological research are shown in table 1. All requirements for work with laboratory animals were followed during the tests.

Cytostatic action of perchlorates N-tolyl-, N-phenyl- and N-hydroxyphenyl-3-oxipyridine (RL-3, RL-175 и RL-S) was evaluated by radiometric method [24]. Results are shown on fig.2 (a; b).

In clinic, we aprobated preparation RL-175 for 5 patients (4 male and 1 female aged from 2 to 65 years old) with acute lymphoblastic leukemia in condition of blast crisis (fig. 2d, 60-70 % blasts in myelogramme), and also 15 patients with thrombocytopenia of different genesis (table 2 and 3).

30 days after taking hormones and traditional chemotherapy (usually, CHOP) all 20 patients were treated by preparation RL-175 in the outpatient setting in hematological department of the North-Ossetian centre for Medical Bioligical problems (Vladikavkaz, Russia). Preparation RL-175 was given per os in dose of 5 mg daily during 30 days (I course). After a 5-day break treatment course was repeated depending on objective and subjective patient's condition.

Authors of this article synthesized the experimental preparations using cheap and available materials. The preparations were preclinically tested in the Oncological Scientific Centre of the Russian Academy of Medical Sciences (Moscow) and in other Russian academic centers.

Physicochemichal and biologic properties of the preparation are described in works [5,6]. LD_{16} for preparation RL-175 when given per os to rats and mice is 1200 mg per 100 g of body mass.

Criteria for evaluation wether preparation RL-175 as a mean of the last chemotherapy line had positive effect was a complete restoration of all clinical hematological and cytological symptoms of desease – normalization of blood hemopoiesis, including thrombocytes, and blast cells content in bone marrow not more than 5% given they have normal cellularity.

Clinical hematological research and exploratory puncture of bone marrow were conducted according to traditional methods. To evaluated results reliability we used Students t test.

Conclusion

Non-cytokine activation by N-substituted 3–oxipyridine salts of pluripotent stem cells' division into myeloid and lymphoid, and then accelerated stimulation of proliferation of early precursors from mixed stem colonies (CFU-GEMM), granulocytic macrophage colonies (CFU-GM), erythroid bursts (BFU-E) till their terminal maturation can substitute a number of linear specific differentiating factors: G-CSF, EPO, TPO and couples anti-gp130 MCA (MCA-agonists B1+12, B1+F1, B-S12) [13].

It is not the most important fact that the above mentioned non-cytokine stimulation of stem cells by N –substituted 3–oxipyridine salts opens new unlimited biotechnological opportunities of their creation. It is phenomenal, that experimental compounds help restore genetic program of stem cells development lost or weakened during ontogenesis and provide its normal realisation, blocking at the same time negative effect of different mutant factors. It is extremely important for Regenerative Medicine.

As you know [25], one of the main functions of gene p53 is to launch programmed cell death when the cell genome is damaged. When p53 is unadequately expressed or mutates, a program of temporary stop of cell cycle is being switched on at the G_1S–stage with the help of protein $p21^{WAFI}$. This factor predisposes to appearance of tumor cells and development of resistance to chemotherapy.

Hwang P., et al [26] revealed a new p53's function in their experiments: this gene plays an important regulatory role between glycolysis and aerobic respiration in malignant cells. Though aerobic glycolysis in tumor cells is considerably reduced, ATP concentration in these cells was comparable with norm. Blast cells' reverse entering of normal cell cycle of proliferation from dormancy (G_1S) through stage of mitosis (MG_2) at patients with ALL could be regarded as a serious evidence in

support of our new conception of cell genome activation, as well as of carcinogenesis model by Hwang P., et al.

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