CHEMICAL COMPOUND RL – 175 – A BREAKTHROUGH IN CONTEMPORARY THERAPY OF DISSEMINATED PROSTATE CANCER

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At present medicinal effect of disseminated hormone dependent prostate gland cancer (DPGC) therapy includes medical castration in combination with antiandrogen application. However the effectiveness of blocking both testicular and adrenal antiandrogen production is little and this does not lead to the DPGC recovery. The average duration of the response to hormonotherapy does not exceed 18 months and the effectiveness of chemotherapy comprises only about 9 %.
In this paper we present the experimental and clinical results of 10 DPGC fatal cases therapy by chemical preparation RL-175 developed by the author. As a result these patients at $T_4N_1M_{1-3}$ stage have been brought to nearly complete tumor remission, to both subjective and objective stabilization of tumorogenic process for 3 and more years. The conception is being discussed of both overcoming and blocking the development mechanisms of this tumor hormonotherapy and chemotherapy resistance under the effect of RL-175, as well as the fundamental possibility of the widest possible use of RL-175 with the preventive purposes.
Key - words: antiandrogens; preparation RL-175; disseminated prostate gland cancer; medical castration.

Introduction

For the last years considerable success in formation of new therapeutic methods and clinical diagnostics of prostate gland cancer (PGC) has been achieved. First of all it regards the results of the European cooperative randomized investigation [l] and the American oncologic group [2-4] as well as [5, 6]. It follows from these papers that nowadays androgen deprivation is one of the most prospective ways of the treatment effectiveness increase of patients with locally invasive PGC. Androgen deprivation includes surgical castration (total or subcapsular orchiectomy) or medical castration. Orchiectomy is considered to be a standard regarding which medical castration is assessed. The latter method includes the use of two preparation groups: estrogens and RH-LH analogues (diethylstilbestrol, gozereline acetate, leuprolide acetate, treptoreline, busereline and others). One kind of castration presupposes the administration of antiandrogens - flutamide (eulexin) and bikalutamide (casodex).

Medicamental castration objectively lowers the level of testosterone to the orchiectomic effect. However these generally accepted methods manifest considerable side effects. Even if they are still relatively effective in the treatment of $T_3N_0M_0$ stage patients, they are not accepted at all with the DPGC ones.

According to the growth rate PGC takes the 2-nd place after melanoma. Among male death causes PGC takes the 2-nd place after lung cancer. When tumor is localized a radical prostatectomy is indicated. However by some data in 50% of cases recurrence follows it [5,6]. In spite of the state programs of PGC early recognition in the developed countries the tumor is detected on the III-IV stages in the most of patients (61%). The absence of such a program in Russia has led to the fact that up to 80% of PGC patients during their primary inspection have already had remote metastases [7].

Thus the DPGC treatment is the most complicated problem. The average life span of PGC patients after surgical and medical castration in the combination with antiandrogens does not prevail 18 months [8], and the effectiveness of chemotherapy regimen of PGC hormone-resistant patients is 9% [9].

On the other hand well-known methods of therapy are not accepted or are of little use in PGC prophylaxis. It seems(it is obvious) that without wide prophylaxis of the population PGC will remain a vital issue for the future. In this connection the searching of new preparations and ways of PGC treatment is

topical. Our work is focused on the attempts to solve the problem.

Patients and Methods

By the authorization of the Ministry of Public Health of Russia № 10-13, July 21, 1991, ten fatal cases aged 50-66 (average age - 58) having the morphologically and cytologically verified diagnosis as PGC of the $T_{3-4}N_1M_{1-3}$ disease stage were picked out for the approbation on a voluntary basis.

Prior to the experiments all the patients had undergone a complete course of the up-to-date therapy. In all the ten cases the said course of treatment had either very little or no effect at all.

RL-175 was administered to all the said patients every day orally on an empty stomach during 30 days. The daily dose of the preparation was 15 mg dissolved in 10 ml of the fruit syrup or in the physiological salt solution.

RL-175 is a heteroaromatic compound. Its physicochemical and biological properties are described in [10].

The preparation effectiveness evaluations after the treatment course completion were: the finger test of the prostate gland; ultra-sound test; the computer tomography and the patient life span after the latest unfavorable prognosis for the disease.

It should be taken into consideration that these ten patients lived in various towns of Russia, all of them were examined and treated in different oncological hospitals. That's why the author couldn't have all the official protocols proving the real effectiveness of the monotherapy with RL-175.

The present article contains the official conclusion of the oncologic dispensary № 4 from the Health Department of Udmurt Republic (Russia). It can be taken as an objective averaged conclusion for ten patients.

Patient S.(1928), a pensioner, lives in Izhevsk, Udmurt Republic (Russia). First he turned for help to the oncological dispensary №4 in Izhevsk in 1988. Based on the biopsy of the prostate gland № 687-88 and № 689-90, the specialists diagnosed the acinose adenocarcinoma with the discomplexation and the far metastasis.

After the general courses of the hormonotherapy and chemotherapy in the III Central Administrative Board of the Ministry of Public Health of the USSR the patient's state improved and the metastatic centers in the bones disappeared. In November 1993 the patient's state sharply became worse. The patient had strong pains in his back, he stayed in bed, lost weight and felt worse. Scintigraphy of the skeleton bones showed the metastases in the area of IX-X ribs on both sides. He also had up to 40% of the bone metastases in the area of the pelvis bones and other places.

The secondary examination of the patient S. in November - December 1993 confirmed the first diagnosis: the prostate gland cancer of $T_4N_1M_{1-3}$ stage with the metastases into the spine and ribs, into the iliac area and possibly into the urinary bladder. With the help of the rectal counter the tumor size was determined as 5.4-5.1cm. The doctors evaluated the patient's state as critical with the nearest unfavorable prognosis.

Then the patient was treated with 4 monotherapy courses based on the preparation RL-175 with the short breaks between the courses. The first course started on February 10, 1994 and lasted till May 10,1994.

Dependence of including [^{3}H]-thymidine, [^{3}H]-uridine and [^{3}H]-L-leucine into the cell of human ovarian carcinoma line from concentration of preparations RL-3, RL-175 and RL-S was made by radiometric method [11]. The results are set out in Fig. 1 (a, b, c).

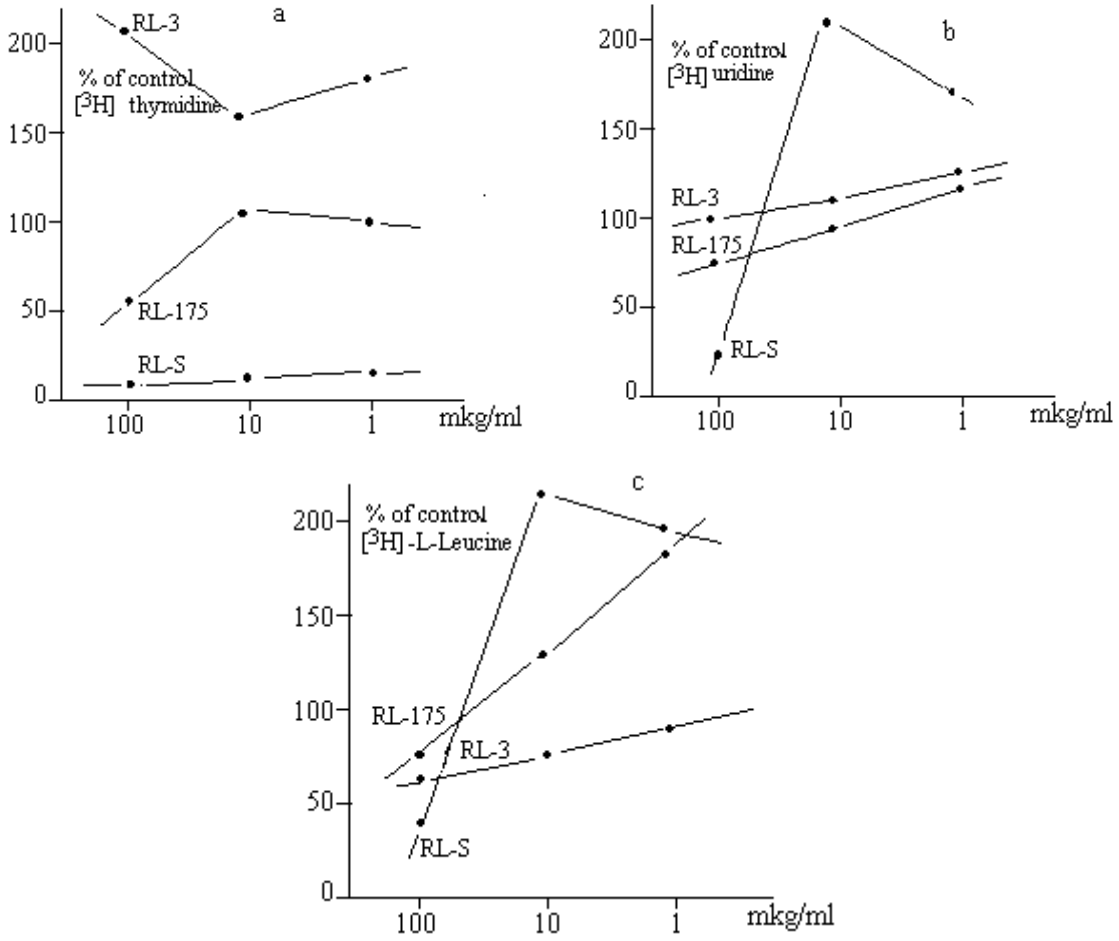


Figure.1. Dependence of including [^{3}H] – thymidine (a), [^{3}H] – uridine (b) and [^{3}H] – L-Leucine (c) into the cell of human ovarian carcinoma line from the concentration of preparations of RL series.

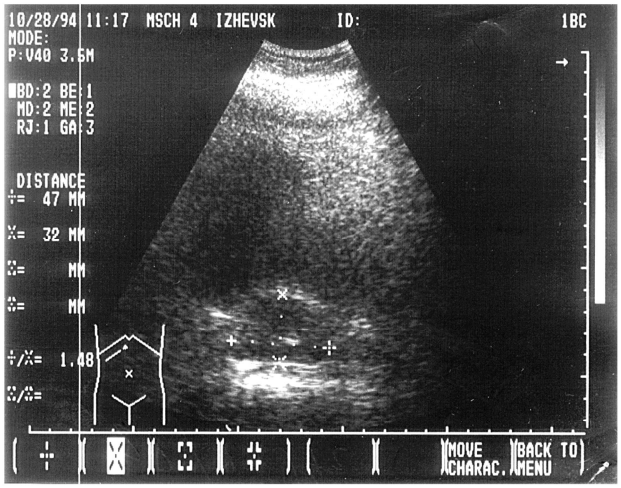
The data resulting from the studies, were processed by using variation statistics Student's t ratio.

Clinical investigations on human subjects were correct in compliance with generally accepted ethic and moral principles in correspondence with the Declaration of 1964 and 1995(revised in Edinburgh, 2000). Into the clinical approbation fatal cases were included on a volunteer basis**.** They gave written consent.

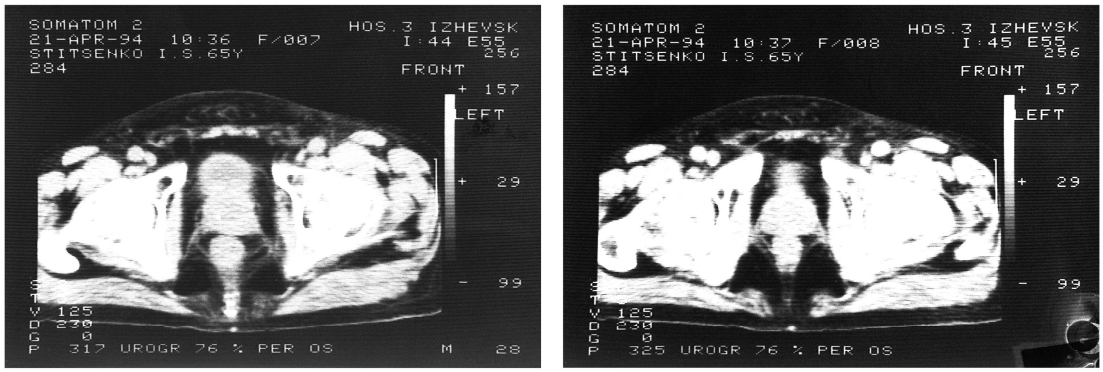
Results

The computer tomography held on April 21, 1994 showed: after the first course of monotherapy on RL-175 there was a moderate decrease of prostate gland in 35x44x50 mm (tumor regression for about 50%); the front contour wasn't clear, closely connected with the urinary bladder, its walls were infiltrated (Fig.2). The computer tomography comparative analysis of the abdominal peritoneum cavity and the small of the back (April 21, 1994 and February 22, 1996) didn't show the negative dynamics of the tumor increase.

The U-sound diagnostics results on September 28, 1994 (Fig.2) showed the gross section size of the prostate gland - 47mm, front-rear area - 32mm the contour was rough, the structure was diffusely nonuniform.



a



b

Figure 2. The upper fig.(a) –U-sound diagnostics of the patient's prostate after 8 months of the RL-175 reception beginnings (tumour size 47x32 mm). The tumour size according to the rectal counter were 54x51 mm prior to the preparation reception. The fig.b – the computer tomography of the prostate after the first course of monotherapy on RL-175 (35x44x50 mm) negative dynamics of the tumour growth was not revealed since 04.21.1994 to 02.22.1996.

Now the patient's general state is quite satisfactory. He is active, has a good appetite, drives a car, goes fishing.

From the very beginning of RL-175 reception the full blockade of the pain has taken place. The examination period is 48 months.

In the experiment we showed that preparations under the code RL-175 accelerate, on 60-80% ($P<0,01$) the oxidation of succinate by rat mitochondria ($1.14x10^{-6}$ mole•s against $0.67x10^{-6}$ mole•s in control) what is adequate at the same time to the acceleration of phosphorylation of ADP in ATP ($ADP+P_i \rightleftarrows ATP$) [12]. By the method of qualitative thin-layer and gas-fluid chromatography there also was revealed increase in vivo in skeletal and cardiac muscles of experimental rats fed by RL-S and RL-175, synthesis ATP up to 3 times regarding control indices.

However by that veritable free energy (ΔG) of ATP hydrolysis in pointed cells of muscles by concentration of ATP, ADP and

P_i correspondingly 40, 0.93 and 8.05mM and significance pH 7.0 and 25° C did not increase ΔG discharged at hydrolysis of energetic value in intact erythrocytes, muscle and rat's liver [13]:

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

where:ΔG^{01} – 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 P_i is carried out in standard thermodynamic conditions - 30.5 kj/mole, then the difference in – 21.4 kj/mole comprises the standard energy (ΔG^{01}) of displacing the preparations RL of the mentioned above balanced system (K_b) in the pool of multifermental complex of respiratory chain in 10^{10} times regarding the balanced (K_b°) uninvolved spontaneous passing of molecule A into B K_b° = $1.15x10^{-3}$;

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

where: for A spontaneously turning into B by G^{01} = - 21 .46 kj/mole
$K_b = 5.62x10^3$, ratio [ATP] / [ADP][P_i] is equal to approximately 500.
Apparently under the influence of RL in 3-key points of respiratory

$$NADH + H \rightarrow \overset{area\,I \uparrow ATP}{\overbrace{E-FMN \rightarrow [F_e - S]_n}} \rightarrow \overset{area\,II \uparrow ATP}{\overbrace{Q \rightarrow b \rightarrow C_1 \rightarrow C}} \overset{area\,III \uparrow ATP}{\overbrace{\rightarrow aa_3 \rightarrow o_2}}$$

chain not three but four molecules are synthesized. We should take into account that equilibrium constant K_b° in the chain is being displaced 10^{40} times, it is considerably higher than the physiological norm in regard to control which is 10^{24} times [13]:

$$ADP + Pi \underset{RL\text{-}175}{\overset{10^{16}\ times}{\rightleftarrows}} ATP$$

In addition to the outstanding bioenergetic function of ATP (moving, active transport, biosynthetic metabolism) one more function is known that is amplification (strengthening) of impulse [13]. In the response to the action of some polypeptide hormones, risk factors, mitogens and cytokines (endogenic irritators) on plasmatic membrane of the animal cell in anaerobic conditions ATP from ADP and Pi is synthesized.

The displacing by the preparations RL-175 and RL-S of thermodynamic equilibrium constant ADP + $P_i \rightleftarrows$ ATP in 10^{16} times in regard to the physiological norm is associated with the considerable synthesis of ATP in the plasmatic membranes of malignant cells in regard to ATP zero concentration in neoplasia [14]. The ATP energy increase in the plasmatic membranes of malignant cells becomes again accessible to the endogene receptors, particularly for the insulin receptors [14,15]. This provides the cascade of mechanisms starting, mechanisms of the cell ingress in to the nornal cycle of the cell proliferation via tirazine kinase receptors activation and via other factors.

Structural dependence of the investigated compounds on the synthesis of DNA and RNA based on inclusions of [^{3}H] - thymidine on [^{3}H] - uridine into the cell of human ovarian carcinoma CaO_Y line was revealed. For example, under the RL-3 influ-

ence synthesis of DNA decreases twice when the dose of preparation is 100 mkg/ml (fig. 1 a, b). This effect of RL-3 on the DNA synthesis is persistent even in case of low concentrations.

Preparation RL-175 in the dose 100 mkg/ml makes partial inhibiting influence on the inclusion speed [^{3}H] - thymidine. Synthesis of DNA increases in doses 10 and 1.0 mkg/ml of the mentioned preparation. In its turn RL-S practically wholly inhibits DNA synthesis and at the same time considerably stimulates RNA synthesis: inclusion [^{3}H]—uridine in the dose of 10 mkg/ml on 214% regarding on the control index.

On Fig. 1c there are presented the data of RL-series preparations influence on the inclusion rate of [^{3}H]-l-Leucine into the cells of human ovarian carcinoma kind. It results from the data that protein synthesis corresponds to the proliferation rate.

Discussion

At present when treating metastatic PGC a number of cytostatic agents (metoxantrone, estramustine, docetaxele, vinblastine, vincristine and others) is used. A therapeutic target for them is Bcl-2, P-glycoprotein, topozomeraze, cytoplasmatic tubes and others. Active search for effective antibodies and rational combinations of drug dosage regimens is being carried out [16].

Specialists hold to the same opinion that the key role in hormonal and chemical resistance of this tumor belongs to genes and antigens controlling apoptosis: p53, PTEN, Bcl-2 and others [15,17-19].

The sensor of DNA damage and cellular cycle damage is gene 53 that codes nucleus protein with molecular mass 53 kD and is able to initiate 2 programs independent of each other:

- temporal failure of cellular circle in G_1S-phase by means of protein p21 inhibiting cyclyne-dependent kinases [17];
- stimulation of apoptosis by the activation of Bax or Bid of Bcl-2 family genes. Pro- and anti-apoptotic action of activated proteins of Bcl-2 family is achieved via the modulation of mitochondria activity.

From the above mentioned experimental and clinical material it may be concluded that under the influence of RL series preparations there takes place a reverse in the mechanisms of the mutarogenic cell ingress into normal mitotic cellular proliferation cycle. The ratio of phases of this cell mitotic cycle $MG_2 > G_1S$ is the measure of gene p 53 activation.

The exclusive role in the apoptosis [15] starting belongs to mitochondria as opposed to hypothesis [20]. Apparently under the influence of RL-175 the apoptotis signal ingress into the nucleus increases parallel with ATP from ADP synthesis growth. The activation of nucleus gene P53R2 that codes the ribonucleotidreductase is achieved in this way. This enzyme is responsible for reparation of mutated DNA.

In the cases, when for some reasons DNA repair is impossible, a cascade of apoptosis mechanisms is started/activated very quickly. This process is accompanied by the outflow of cytochrome c, ATP, Ca^{2+} apoptosis-inducing factor into cytozole via metachannels due to the Bcl-2 family protein activation. Cytochrome c activates caspase 9 which in its turn activates killer caspase 3. Thus the signal way of apoptosis caused by DNA impair is finished.

Thus the possibility of gene p53 activity correction up to the norm and starting the apoptosis mechanisms via family Bcl-2 gene activation under the preparation RL-175 influence is a serious factor in overcoming the resistance barrier of this tumor to hormonotherapy and chemotherapy. If the average DPGC patients life span is rather low, then a 4-year almost complete remission of 10 fatal cases of $T_4N_1M_{1-3}$ stage is evidence of the near victory in the fight with this severe disease.

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