

Chrono- and Immunocorrection of Inflammatory Disorders of Internal Reproductive Organs in Women of Reproductive Age

G. I. Litvinenko, A. V. Shurlygina, T. I. Dergacheva*,
E. V. Mel'nikova, and V. A. Trufakin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 159, No. 1, pp. 71-74, January, 2014
Original article submitted December 23, 2013

We compared the effectiveness of immunomodulators used in the treatment of patients with chronic salpingitis and oophoritis with or without changes in succinate dehydrogenase (SDH) activity in blood lymphocytes at incubation with the drug. Diurnal variations in individual reaction of SDH in blood lymphocytes to thymalin or ridostin were revealed. In the groups of women receiving ridostin or thymalin during the reaction of lymphocyte SDH to it, improvement of clinical laboratory and immunological parameters was observed in the majority of the patients and no effect was found in a lesser group of patients than in the groups treated with drugs during the absence of lymphocyte SDH reaction thereto. The timing of the presence of SDH reaction to drugs in the immunocompetent cells makes it possible to set the optimal daily regime of their application and to select a drug that would be most effective in each particular case.

Key Words: *chronic inflammation; immunomodulation; lymphocytes; chronotherapy*

Chronic inflammatory disorders of internal reproductive organs markedly affecting the health of women of reproductive age are the most common cause of reproductive disorders in women and adversely affect the health of the nation [4,10]. This pathology alters immune reactivity with disturbances in the systems of T- and B-cell immunity and decrease in the phagocytic activity of monocytes and neutrophils [5], which contributes to the recurrence of inflammation and resistance to standard antibacterial and anti-inflammatory therapy. Therefore, the use of immunomodulators in the complex treatment of chronic inflammatory processes is a reasonable and necessary measure [4,10]. However, the effectiveness of immunocorrection is still insufficient (45-50%). In light of this, the improvement of the efficiency of immunocorrection is a pressing problem that can be solved by using chronobiological approach.

Chronopharmacology is a promising trend in modern pharmacology investigates the role of the time factor in the effects of drugs. Chronopharmacological approach allows optimization of conventional therapy [11,13,14]. We have experimentally shown that application of thymus preparations, hydrocortisone, IFN- γ , and IL-2 leads to different results depending on the time of day of administration [3,6,8,9]. Choosing the diagnostic and prognostic criterion for identification of the optimal time for drug application is an unsolved problem. We used changes in succinate dehydrogenase (SDH) activity in blood lymphocytes after incubation with the drug as a "marker". Since the level of energy metabolism in lymphocytes and activity of redox enzymes are closely related to their functional characteristics [2], this test gives an indication of the response of lymphoid cells to the drug, *i.e.* of their "sensitivity" to the drug [1].

The purpose of this work was comparative study of the clinical and immunological effectiveness of immunomodulators used in the treatment of patients with chronic salpingitis and oophoritis depending on the presence or absence of changes in SDH activity in patients after incubation with the drug.

Research Institute of Physiology and Fundamental Medicine; *Research Institute of Clinical and Experimental Lymphology, Novosibirsk, Russia. **Address for correspondence:** litv_g_i@mail.ru.
G. I. Litvinenko

MATERIALS AND METHODS

The study included 98 women suffering from chronic salpingitis and oophoritis in the acute stage (ICD-10) and 38 healthy women aged 18 to 35 years. Groups of patients were comparable by age and duration and clinical course of the disease. All women underwent clinical and laboratory examination.

The treatment consisted of antibiotic therapy (injection of a broad-spectrum antibiotic under the vaginal mucosa; the drug was chosen taking into account microflora sensitivity [3]), vitamin therapy, and, in the absence of contraindications, physiotherapy.

Thymalin or ridostin were applied in order to correct the immune status. Ridostin in a dose of 8 mg was administered once a day every other day (up to 5 intramuscular injections). Thymalin was administered intramuscularly by 1 ml once day for 5 days.

Immediately before immunomodulator administration, SDH reaction thereto was determined at 09.00 and 17.00. Thymalin or ridostin were administered randomly at 09.00 or 17.00 without taking into account the reaction results. The presence or absence of SDH reaction to the drug during its administration was evaluated retrospectively. Clinical and laboratory examinations were performed 17-18 days after treatment and assessed the effectiveness of therapy by the following criteria: "improvement" (improvement of health, pain relief and no discharge, disappearance of infiltrative changes in the appendages, normalization of

hematological, biochemical, and bacterioscopic indicators) and "no effect" (aggravation or stabilization of clinical and laboratory signs of inflammation). Immune status and activity of SDH and lactate dehydrogenase (LDH) were examined before and after the treatment.

Subpopulation ratio of blood immunocompetent cells was assessed in the morning on a FACSCalibur flow cytometer using FITC- and phycoerythrin-labeled monoclonal antibodies to lymphocyte markers CD3, CD4, CD8, CD16, CD20, and HLA-DR (Sorbent). SDH and LDH activities were measured also in the morning by cytochemical quantitative method with p-nitrotetrazolium violet [2]. SDH response to thymalin and ridostin was evaluated at 09.00 and 17.00 by changes in enzyme activity after incubation of blood samples with the drug *in vitro* [1].

The significance of intergroup differences was evaluated by nonparametric Mann-Whitney *U* test, frequency analysis was conducted using χ^2 test.

RESULTS

We revealed increased numbers of NK cells (CD16⁺ lymphocytes), reduced number of activated monocytes (HLA-DR⁺ monocytes) in the blood of patients before treatment in comparison with healthy women (Table 1), which attested to disorders in the phagocytic component of the immune system associated with frequent and prolonged bacterial infections as well as chronic infectious and inflammatory processes [5,12].

TABLE 1. Percentage of Various Subpopulations of Immunocompetent Cells and SDH and LDH Activity in Healthy Women and Patients with Chronic Salpingitis and Oophoritis before and after Treatment with Ridostin or Thymalin in Various Regimens ($M \pm SE$)

Parameter	Healthy women (N=38)	Sick women		
		before treatment (N=98)	after treatment	
			R- (N=49)	R+ (N=49)
CD3 ⁺ , %	65.50±1.26	64.4±2.8	71.1±2.2*	70.4±1.7*
CD4 ⁺ , %	36.70±0.81	32.3±3.0	41.4±1.5*	38.6±1.6
CD8 ⁺ , %	26.20±1.78	30.2±2.0	34.8±1.9*	25.5±0.9
CD16 ⁺ , %	9.30±1.59	14.9±2.0*	6.6±1.1**	8.1±1.1*
CD20 ⁺ , %	11.40±0.62	8.9±1.7	7.2±0.7*	8.4±0.6*
HLA-DR ⁺ monocytes, %	84.90±2.77	51.2±3.9*	43.9±4.5**	72.3±2.6*°
CD4 ⁺ /CD8 ⁺	1.47±0.20	1.4±0.2	1.3±0.2	1.3±0.2
SDH (granules per lymphocyte)	16.7±2.2	11.2±0.9*	10.3±1.1*	23.4±3.9
LDH (granules per lymphocyte)	14.2±2.4	10.7±1.8*	12.3±3.2*	14.8±2.7

Note. R-, administration of the drug in the absence of SDH reaction to it; R+, administration of the drug in the presence of SDH reaction to it. $p < 0.05$ in comparison with *healthy women, **data before treatment, °R-.

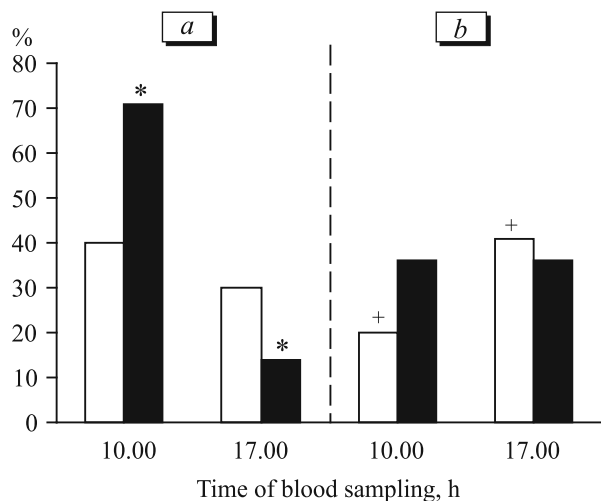


Fig. 1. Diurnal variations in SDH reaction to thymalin (a), ridostin (b) in healthy women (light bars) and patients with chronic salpingitis and oophoritis (dark bars). Vertical axis, proportion of patients with SDH reaction to incubation with thymalin or ridostin. Values significantly different at $p < 0.05$ (χ^2 criterion) are marked with identical symbols.

Changes in the redox metabolism of the lymphocytes were expressed in reduced activity of SDH and LDH that can be regarded as functional impairment of immune cells [2]. Diurnal variations in SDH reaction to thymalin and ridostin were found in healthy women and in the patients (Fig. 1). The time of SDH reactions to the drugs varied. Some patients showed it in the morning, the others, in the evening, and some persons exhibited the presence or absence of the reaction at both time points. In the latter case, any time of administration was regarded as coinciding or not coinciding with the presence of SDH reaction. Moreover, the reaction could be present for one drug and lack for another one. This suggests the need in individual research to customize the choice of the most appropriate immunocorrector and determine the optimum time of application.

Evaluation of clinical and laboratory parameters of the efficiency of different regimens of immunomodulator administration gave the following results. In the groups of women treated with ridostin or thymalin during the lack of SDH reaction thereto, improvement after treatment was noted in a significantly lower proportion of patients, and no effect, in significantly greater proportion of the patients than in the groups treated with drugs during SDH reaction thereto (Fig. 2).

The relative number of T cells ($CD3^+$) was increased in all groups after the treatment. Patients administered with ridostin or thymalin during the lack of response of blood lymphocytes to the drug increased the number of T lymphocytes $CD4^+$ and $CD8^+$ after the treatment, reduced the number of B lymphocytes, and the percentage of HLA-DR⁺ monocytes remained reduced in comparison with healthy donors. Activity

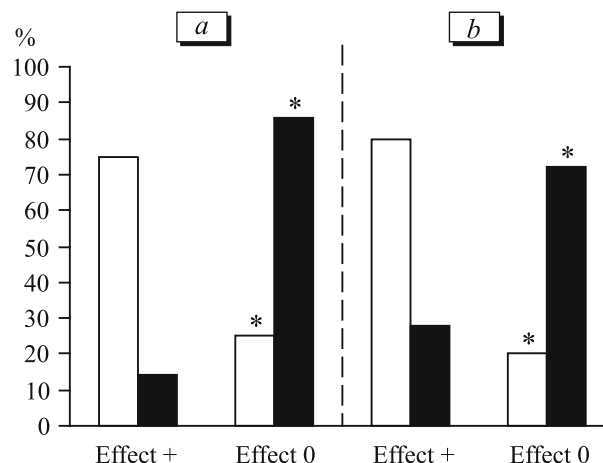


Fig. 2. Clinical effectiveness of different modes of daily application of thymalin (a) and ridostin (b) in the treatment of chronic salpingitis and oophoritis. Vertical axis, proportion of patients with improvement or no effect, horizontal axis, presence (effect +) or absence (0 effect) of the effect of treatment. Light bars, the time of drug administration coincides with the time of SDH reaction on it; dark bars, does not coincide with the time of SDH reaction on it. * $p < 0.05$ (χ^2 criterion) in comparison with the presence of the effect.

of SDH and LDH remained reduced. In the group of patients administered with the drug during SDH reaction thereto, the percentage of NK cells ($CD16^+$) decreased to control values and the percentage of HLA-DR⁺ monocytes elevated while SDH and LDH levels were normalized (Table 1). Application of chronotherapeutic treatment regimen reduced the number of injections of ridostin from 5 (according to the instructions of Ministry of Health of the Russian Federation) to 3 as well as to reduce the dose of thymalin 2-fold maintaining a positive clinical and immunological effects of treatment.

Calculation by the method [7] has shown that the reaction of SDH on immunomodulator (ridostin and thymalin) allows to predict the effectiveness of a particular drug. The high sensitivity and specificity of the method was marked, as well as a high degree of reliability of the forecast of negative result, which makes this method valuable to prevent unreasonable use of the drug (Table 2).

TABLE 2. Prognosis of Efficiency of Immunomodulators (Thymalin and Ridostin) by SDH Response to These Preparations (calculated by the method [7])

Parameter	Value, %
Sensitivity of the test	82
Specificity	86
Positive prognosis	60
Negative prognosis	95

Thus, immunomodulatory therapy conducted by chronotherapeutic principles significantly improves the clinical and immunological effectiveness of treatment of chronic salpingitis and oophoritis. The timing of SDH reaction of immunocompetent cells to the drugs allows to set the optimal daily regime of their application and to select a drug that would be most effective in each case or to avoid undue application of the preparation, which should contribute to the individualization of treatment taking into account the features of temporal organization of the physiological functions and the course of the disease.

REFERENCES

1. A. V. Shurlygina, T. I. Dergacheva, V. S. Dergachev, *et al.*, *Author's Certificate No. 1780752, Method of Immunotherapy*, *Byull.*, No. 46, December 15, 1992.
2. L. M. Kurtasova, A. A. Savchenko, and V. T. Manchuk, *Metabolic Aspects of Immune Disorders in Children with Respiratory Diseases* [in Russian], Novosibirsk (2001).
3. T. I. Dergacheva, N. V. Anastasieva, V. A. Trufakin, *et al.*, *Patent RF No. 2145891, Method of Treatment of Patients with Chronic Adnexitis, Infectious-Toxic Variant of Nonspecific Etiology*, *Byull.* No. 9, February 27, 2000.
4. V. N. Serov and M. V. Tsaregorodtseva, *Akush. Gin.*, No. 1, 32-33 (2009).
5. L. A. Trunova, O. V. Tikhonova, A. V. Efremov, and A. N. Trunov, *Byull. Sib. Otdel. Ross. Akad. Med. Nauk*, No. 1, 91-95 (2001).
6. V. A. Trufakin, A. V. Shurlygina, T. I. Dergacheva, and G. I. Litvinenko, *Bull. Exp. Biol. Med.*, **119**, No. 2, 173-175 (1995).
7. R. Fletcher, C. Fletcher, and E. Wagner, *Clinical Epidemiology. Evidence-Based Medicine* [in Russian], Moscow (1998).
8. A. V. Shurlygina, T. I. Dergacheva, I. G. Kovshik, *et al.*, *Byull. Sib. Otdel. Ross. Akad. Med. Nauk*, No. 5, 66-72 (2008).
9. A. V. Shurlygina, O. T. Kudaeva, O. P. Kolesnikova, *et al.*, *Byull. Exp. Biol. Med.*, **156**, No. 1, 73-77 (2013).
10. E. C. Barbiero, M. G. Sartori, and M. J. Girao, *Int. Urogynecol. J. Pelvic Floor Dysfunct.*, **14**, No. 5, 331-334 (2003).
11. N. Burioka, *Nihon Rinsho*, **71**, No. 12, 2146-2152 (2013).
12. C. J. Harvey, R. K. Thimmulappa, S. Sethi, *et al.*, *Sci. Transl. Med.*, Vol. 3, N 78, 78ra32 (2011).
13. G. Kaur, C. Phillips, K. Wong, and B. Saini, *Int. J. Clin. Pharm.*, **35**, No. 3, 344-358 (2013).
14. E. Ortiz-Tudela, A. Mteyrek, A. Ballesta, *et al.*, *Handb. Exp. Pharmacol.*, **217**, 261-288 (2013).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.