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RECURRENT HERPES SIMPLEX: EVALUATION OF INTERFERONOGENESIS AT THE LOCAL AND SYSTEMIC LEVELS

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In this article, the authors propose the results of the investigation of the interferon–alpha synthesis dynamics at the local and systemic levels in patients with recurrent herpes simplex virus, analyzed the effect of different types of therapy on the interferon–alpha synthesis and established the clinical efficacy depending on the therapy received. It was found that patients with mild herpes simplex after antiviral therapy and during follow–up showed normalization of the interferon–alpha level. The results indicate the stabilization of antiviral protection in patients who received antiviral therapy (acyclovir and/or inosine pranobex) and the possible further persistence of the virus in patients treated by acyclovir and/or placebo. The clinical efficacy of antiviral treatment of patients with recurrent herpes simplex virus was established: with a mild course of infection after treatment it was 73.5 %, during follow–up – 85.8 %; with a medium to severe and a severe – after treatment – 64.0 %, during follow–up – 70.4 %.

Keywords: herpes simplex virus, interferon–alpha, antiviral therapy, interferon refractoriness

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РЕЦИДИВУЮЧА ПРОСТА ГЕРПЕСВІРУСНА ІНФЕКЦІЯ: ОЦІНКА ІНТЕРФЕРОНОГЕНЕЗУ НА МІСЦЕВОМУ ТА СИСТЕМНОМУ РІВНЯХ

У даній статті авторами запропоновані результати дослідження динаміки синтезу інтерферону–альфа на місцевому та системному рівнях у хворих на рецидивуючу герпесвірусну інфекцію 1/2 типу, проаналізовано вплив на синтез інтерферону–альфа різних видів терапії та встановлено клінічну ефективність залежно від отримуваної терапії. Виявлено, що у пацієнтів із легким перебігом герпесвірусної інфекції 1 типу після застосування противірусної терапії та у віддалений період відзначалася нормалізація рівня інтерферону–альфа, на відміну від групи пацієнтів, яка отримувала плацебо. Отримані результати свідчать про стабілізацію противірусного захисту в пацієнтів, які отримували противірусну терапію, а саме ацикловір і/ або інозин пранобекс та про можливу подальшу персистенцію вірусу в організмі в пацієнтів, які отримували ацикловір і/або плацебо. Була встановлена клінічна ефективність противірусного лікування хворих з рецидивуючою герпесвірусною інфекцією 1/2: у хворих з легким перебігом після лікування становила 73,5 %, у віддалений період – 85,8 %; у хворих з середньо – тяжким та тяжким після лікування – 64,0 %, у віддалений період – 70,4 %.

Ключові слова: герпесвірусна інфекція 1/2 типу, інтерферон–альфа, противірусна терапія, інтерферонорефрактерність

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Herpes simplex virus (HSV) is included in the group of diseases that determine the future of infectious pathology according to the WHO Regional Office for Europe [4, 5, 7]. Infection and incidence of herpes virus in the general population are faster than the growth rate of the Earth's population and determine not only the medical but also the huge social significance of the problem [3]. Typically, herpes virus infection is caused by herpes simplex viruses – HSV–1 and HSV–2, antibodies to which are found in 90–99 % of the adult population of the planet [2, 7]. In a healthy person, virus replication is prevented by the immune system; viruses persist in the nerve ganglia remaining in a latent state throughout life [1]. However, in cases of a weakened immune system, latent herpes acquires an active form with frequent relapses and long– term manifestations, which is associated with the immune system disorder – impaired antiviral protection, followed by possible hyperactivation of some parts of the immune system and its dysregulation [2].

The most common clinical manifestations of herpes simplex virus are those observed on the skin and mucous membranes. Yet, the analysis of the data of the long–term study of HSV has proved that herpes simplex affects the whole organism, not only skin and mucous membranes and is an immune–related disease [6].

HSV 1/2, like other herpes viruses, is endowed with opportunistic properties, so the reactivation of this pathogen usually occurs in persons with congenital immunosuppression, or secondarily in immunosuppressed patients who have certain acquired immunodeficiency disorders [11], for example, people with HIV, cancer, systemic autoimmune diseases, organ transplant recipients. These patients receive long–term cytostatics, radiation therapy, monoclonal antibodies, which can cause secondary immunosuppression and mediate the formation of herpes virus neuroinfection [10].

It is known that the formation of an adequate antiviral immune response is possible only with an excellent performance of the innate and acquired, humoral and cellular components of the immune system.

The interferon (IFN) system, in particular IFN-alpha (IFN-α), plays a significant role in innate antiviral protection of the body. Induction of interferogenesis is performed almost immediately after viral infection, and in 30–40 minutes the signs of genomic activation of production cells appear. Synthesis by IFN-α cells and its secretion into the environment is observed immediately after the induction stage. After 2–3 hours, an increased concentration of functionally active IFN-α is detected in peripheral blood, and after 6–8 hours, the concentration of IFN-α reaches its maximum. IFN-α induced cascade of intracellular reactions leads to inhibition of replication of a wide range of viruses. Thus, during the acute phase of viral infections, the IFN-α concentration increases and correlates with the increase of the virus titer, and during the convalescence, the concentration of IFN-α decreases to the normal level [8]. IFN-α plays a key role in local and systemic antiviral protection, since the innate nonspecific immune response inhibits virus replication at an early stage and allows for the short–term mobilization of an adaptive immune response, which is necessary for the complete elimination of the infectious agent.

The problem of the effective treatment of patients with recurrent herpes simplex, and the safety of immunotropic drugs with mediated antiviral action remains relevant.

The purpose of the work was to perform analysis of the IFN-α synthesis dynamics at local and systemic levels in patients with recurrent herpes simplex and evaluation of the impact of different types of therapy on its synthesis.

Material and methods. The study involved 200 patients who underwent outpatient treatment in the Lviv Regional Clinical Diagnostic Center and Lviv Regional Clinical Hospital – clinical bases of the Department of Clinical Immunology and Allergy of the Danylo Halytskyi Lviv National Medical University. These were male and female patients (the vast majority were women – 84.2 %), mainly aged 25–44 (66.8 %). All patients were diagnosed with acute recurrent herpes virus type I and II, that is, the patients were assigned to the immunocompromised group. The diagnosis was verified on the basis of medical history, clinical findings of recurrent herpes simplex virus, laboratory criteria (determination of HSV 1/2 DNA in blood or/and saliva or/and scraping of the lesions; serum diagnostics: detection of HSV1/2 IgM and HSV1/2 IgG antibodies in serum). The control group included 44 practically healthy persons.

Patients were stratified into subgroups according to the severity of the disease. The severity of recurrent herpes virus was determined by the number of relapses per year, as well as by the presence of neurological (peripheral neuritis), visceral (HSV hepatitis, cystitis, urethritis, pneumonia) and other complications, as well as atypical disease (edema syndrome, allergic dermatitis, etc.). Patients who had 1–2 relapses per year without visceral and neurological complications were included in the group with a mild disease – 108 patients (55.1 %), patients with 3–5 relapses per year with complications or atypical disease were classified into a group with a moderate to severe disease. The group of patients with a severe disease included patients who had 6 or more relapses per year, as well as complications, especially neurogenic, systemic autoimmune complications. The combined group of patients with a moderate to severe and a severe disease consisted of 88 patients (44.9 %).

The study had several stages: screening, treatment (90 days) and follow-up up to 180 days and included 5 visits. The total duration of participation in the study was 6 months.

Patients were randomized in the treatment groups in a ratio of 1:1 depending on the use of Inosine pranobex (Novirin, produced by “Kyiv Vitamin Plant”, Ukraine). In the groups, patients were stratified into two subgroups according to the severity of herpes virus: the first subgroup consisted of patients with a mild disease, the second subgroup included patients with a moderate to severe and a severe disease. Patients of subgroup 1 received a therapeutic dose of inosine pranobex 100 mg/kg/day 1 to 14 day, and subsequently a maintenance dose of 50 mg/kg/day – 15 to 90 days. Patients of subgroup 2 received 100 mg/ kg day of inosine pranobex and 1600 mg/day of acyclovir for 14 days, after that a maintenance dose of inosine pranobex 50 mg/kg/day up to 3 months.

All patients were tested for IFN-α in blood and saliva. The IFN-α level in saliva was determined before the start of treatment, during the first seven days of taking the medicine, after the end of treatment (90 days) and during follow-up (180 days). In blood, the concentration of IFN-α was determined before the start of treatment, on 3, 5, 7 day of receiving the medicine, after 90 and 180 days from the beginning of treatment. Test for concentration of IFN-α in serum and saliva was performed using the method of enzyme immunoassay according to the guidelines attached to the standard sets of reagents “alpha-interferon-IFA-BEST” (“Vector Best”, Russian Federation). These studies were conducted in the immunological laboratory of the Danylo Halytskyi LNMU.

The methods of descriptive statistics are used to describe the initial state of the main and control groups (for quantitative indicators – n, arithmetic mean and its standard deviation). For quantitative indicators, the normality of data distribution in groups was checked using the Shapiro–Wilk test. In the vast majority of cases, the presence of a Gaussian distribution is established. Comparison of groups is carried out by means of Student's t–test for independent samples or by means of Paired Student's t – test.

In the case of a non–Gaussian distribution, the comparison of groups was carried out using the Mann–Whitney or McNemar's test. The null hypothesis is rejected at p<0.05.

Results of the study and their discussion. A comparative analysis of the IFN-α level in blood and saliva has been performed in healthy persons and patients with recurrent HSV 1/2, in particular with mild, moderate and severe course (fig. 1).

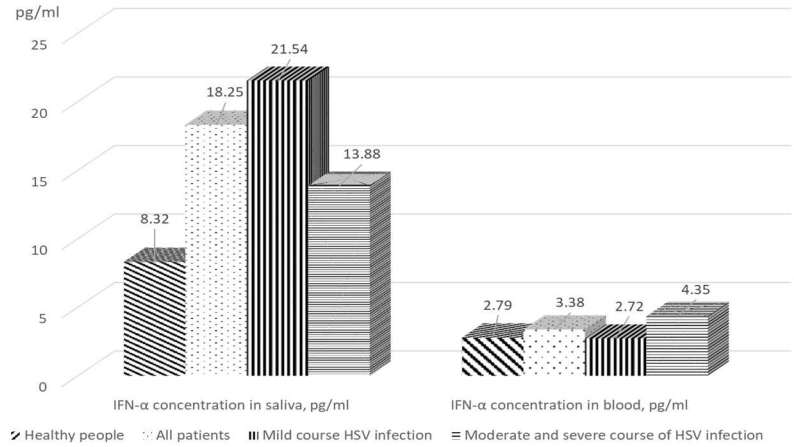

Fig. 1 Comparative analysis of IFN-α levels in saliva and blood in patients with recurrent HSV1/2 with a mild, moderate to severe and severe disease compared with healthy persons

Figure 1 shows that in the mixed group of patients with different degrees of severity of recurrent HSV1/2 before treatment, the IFN-α level in saliva was 18.25±15.78 pg/ml, which significantly differed from that of healthy persons (8.32±4.98 pg/ml, p<0.05). In turn, in patients with mild recurrent HSV1/2 before treatment, the IFN-α level in saliva was 21.54±11.02 pg/ml and was significantly different from that of healthy persons (8.32±4.98 pg/ml, p<0.05) and was significantly higher than in patients with a medium to severe and a severe infection (13.88±17.18 pg/ml, p<0.05). Thisndicates i activation of IFN-α synthesis and a certain depletion of the interferon pool in saliva in patients with recurrent herpes virus.

The concentration of IFN-α in the blood before treatment was higher in the group of patients (3.38±1.18 pg/ml, p<0.05), in particular in patients with mild (3.49±1.12 pg/ml, p<0.05) and moderate to severe (5.12±2.21 pg/ml, p<0.01) recurrent HSV1/2 compared to healthy persons (2.00±0.61 pg/ml). This indicates that the potential of IFN-α genesis in the blood during frequently recurring herpes virus infections is more preserved.

Thus, the obtained data indicate that during the stimulation of IFN-α synthesis as a response to activation in case of recurrence of herpes simplex virus, the concentration of IFN-α rises, although with different intensity in saliva and blood depending on the severity of the disease.

Table 1 shows that the concentration of IFN-α in saliva before treatment in the group of patients who received both inosine pranobex and inosine pranobex +acyclovir (16.57±4.07 pg/ml) and placebo and placebo+acyclovir (19.54±6.56 pg/ml) was significantly higher (p<0,05) compared to healthy persons (8.32±2.98 pg/ml).

Table 1

Comparative analysis of IFN-α in saliva and blood in healthy persons and groups of patients treated by inosine pranobex and inosine pranobex+acyclovir (the first mixed group) and placebo and placebo+acyclovir (the second mixed group) before treatment, after treatment and during follow–up (M±σ)

Saliva						
Groups / Research period	**Healthy**	**Inosine pranobex and inosine pranobex+ acyclovir (mixed group)**	**Placebo and placebo+acyclovir (mixed group)**	P 1-2	P 1-3	P 2-3
Before treatment	8.32±2.98	16.57±4.07	19.54±6,56	<0.05	<0.05	>0.05
After treatment		5.86±1.62	12.03±3,53	<0.05	<0.05	<0.05
Follow-up		6.83±1.78	7.76±2.51	<0.05	<0.05	>0.05
Blood						
Groups / Research period	**Healthy**	**Inosine pranobex and inosine pranobex+ acyclovir (mixed group)**	**Placebo and placebo+acyclovir (mixed group)**	P 1-2	P 1-3	P 2-3
Before treatment	2.00±0.31	4.29±1.77	4.05±1.74	>0.05	>0.05	>0.05
After treatment		2.19±1.22	3.99±1.06	>0.05	>0.05	<0.05
Follow-up		1.8±0.89	3.91±1.03	>0.05	>0.05	>0.05

In the group of patients treated with inosine pranobex and inosine pranobex+acyclovir, the concentration of IFN-α decreased after treatment (5.86±1.62 pg/ml) and stabilized during follow–up (6.83±1.78 pg/ml), which was lower than before the treatment of the group and probably did not differ from that of healthy persons. In patients who received placebo and placebo+Acyclovir, the concentration of IFN-α in the saliva of these patients decreased: during treatment and after treatment it was 12.03±3.53 pg/ml and was significantly different than in the previous group. During follow–up, the concentration of IFN-α practically normalized (7.76±2.51 pg/ml), which was significantly lower than before treatment.

The concentration of IFN-α in the blood of patients with recurrent HSV1/2 who received inosine pranobex and inosine pranobex+acyclovir was significantly higher compared to healthy persons (4.29±1.77 pg/ml 2.00±0.31, respectively) and normalized after treatment and during follow–up. In the group of patients receiving placebo and placebo+acyclovir, the level of IFN-α in the blood compared with healthy persons was significantly ($p<0.05$) higher before treatment (4.05±1.74 pg/ml) and during follow up (3.91±1.30 pg/ml).

Thus, in patients with recurrent HSV 1/2 treated with inosine pranobex and inosine pranobex+acyclovir, the concentration of IFN-α before treatment was by 1.99 times higher in saliva and 2.14 times in blood, compared with healthy persons. After treatment and during follow–up, the level of IFN-α in the studied media did not differ from healthy persons.

If we summarize the data on the patients with recurrent HSV1/2 who received placebo and placebo+acyclovir, the concentration of IFN-α in blood and saliva was high, which may be associated with the subsequent persistence of the virus in the body.

To determine interferon refractoriness we conducted a comparative analysis of the concentration of IFN-α in blood and saliva before treatment, during the first seven days of treatment, 45–52 days of treatment, 90 and 180 days from the beginning of treatment in patients with recurrent HSV1/2 with a mild, moderate to severe and severe disease, receiving different treatment regimens (inosine pranobex and inosine pranobex+acyclovir and placebo and placebo+acyclovir), see fig. 2.

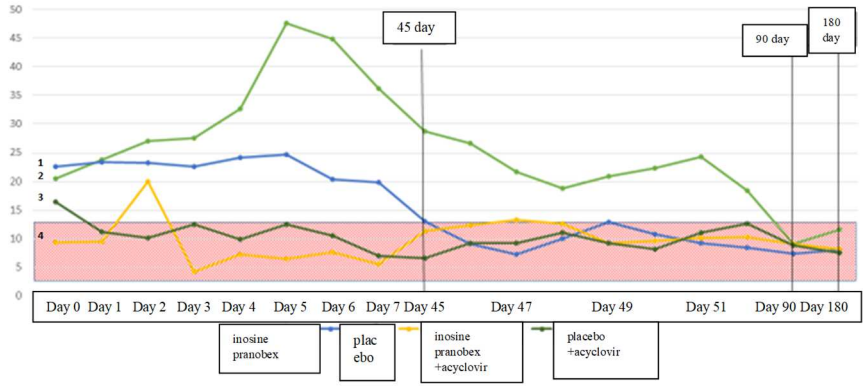

Fig. 2. Comparative analysis of the IFN-α concentration in saliva before treatment, after treatment and during follow–up period

Figure 2 shows that the IFN-α level before treatment, the initial level of IFN-α in the saliva of patients of experimental and control groups did not differ significantly. On the second day in the saliva of patients of the experimental group (green color), who received inosine pranobex, its concentration began to increase and fluctuated within 23.74–47.58 pg/ml for seven days. Starting from day 45, the IFN-α level in saliva began to gradually decrease (from 28.88±5.26 pg/ml to 18.41±8.0 pg/ml. On day 90, its concentration decreased by 2 times and amounted to 9.11±5.44 pg/ml, on day 180 it did not differ significantly from the previous figure (11.50±4.24 pg/ml). In patients who received inosine pranobex+acyclovir (yellow color), the concentration of IFN-α in saliva during the first seven days was significantly lower and ranged between 7.28–4.17 pg/ml with a maximum peak on the second day (19.3±5.26 pg/ml). Starting from day 45, the concentration of IFN-α began to increase and by day 52 was kept within 13.3–9.13 pg/ml, and on day 90 and 180 decreased and did not differ significantly from that in healthy persons (9.10±6.06 pg/ml and 8.24±5.14 pg ml, respectively). In patients treated with placebo (blue color), the IFN-α level in saliva was high (24.72–19.9 pg/ml) only the first seven days. Over the period of 45–52 days, the IFN-α level in the saliva of these patients gradually decreased, fluctuating within 13.04–8.04 pg/ml, reached the level of healthy persons on day 90–7.40±4.67 pg/ml and on day 180–8.01±3.94 pg/ml. In patients treated with placebo+acyclovir (brown color), the concentration of IFN-α in saliva in the first seven days of treatment was probably lower than in patients treated with inosine pranobex, ranging from 12.46–6.91 pg/ml. During the period of 45–52 days, its concentration remained stable, slightly increased on day 51 and day 52 (up to 12.55±6.61 pg/ml) and had an unambiguous downward trend on day 90 (8.74±5.56 pg/ml) and on day 180 (7.49±6.12 pg/ml).

Thus, the patients treated with inosine pranobex had the longest and the highest increase in the IFN-α level in saliva, which normalized after treatment and did not decrease during follow–up. In patients who received inosine pranobex+acyclovir, the concentration of IFN-α in saliva was lower compared to patients who received inosine pranobex, but its concentration gradually began to normalize during treatment and reached the level of healthy persons on day 90, remaining stable during follow–up. The results indicate the interferon regulatory effect of inosine pranobex in patients with recurrent HSV1/2 affected by the absence of interferon refractoriness.

We have also established the clinical efficacy of the proposed treatment of patients with recurrent HSV, which was evaluated on the basis of the dynamics of the number of relapses and specific clinical manifestations: with a mild disease after treatment, it was 73.5 %, during follow–up – 85.8 %; in patients with a medium to severe and severe disease after treatment – 64.0 %, during follow–up – 70.4 %. The

overall efficacy (including clinical, virological and immunological parameters) of all patients treated with inosine pranobex after treatment was 62.2 % and during follow-up – 72.3 %.

The data we obtained can be compared with the data in other studies. Japanese scientists conducted a similar study, which aimed to evaluate the effectiveness and tolerance of Inosine pranobex in the treatment of recurrent herpes simplex virus (labial and genital forms) in comparison with Acyclovir. The study group consisted of 144 patients with labial form and 144 patients with genital forms of herpes simplex virus. In each group, patients were further divided into two groups depending on the recommended therapy. In the first group patients received 4 g/day of Inosine pranobex (1 g four times daily) and 1000 mg/day of placebo (200 mg 5 times a day); the second one – 1000 mg/day of Acyclovir (200 mg 5 times a day) and 4 g/day of placebo (1 g four times daily). The treatment period was 7 and 5 days for labial and genital herpes virus, respectively. The efficacy of treatment was assessed on the 3rd and 5th/7th days of treatment. As a result, the efficacy of treatment in patients with the labial form of herpes simplex virus treated with Inosine pranobex and Ayclovir was almost the same. In the group of patients with genital herpes, the efficacy of treatment did not have a significant difference [12].

Due to the wide and frequent use of Acyclovir (ACV) for the treatment of herpes simplex virus and its availability, it is advisable to pay attention to the concept of "Acyclovir resistance". The prevalence of Acyclovir resistance in patients with herpes simplex virus is usually low in immunocompetent persons (<1 %) but significantly higher in immunocompromised individuals (4–14 %). This difference demonstrates the key role of the host immune system in controlling herpes simplex virus [9]. At this stage, there is a need to continue research related to the study of the effectiveness and safety of antiviral drugs with direct and indirect effects on the activity of viruses, especially herpes simplex virus, given its impact on the immunocompromised cohort of the population.

Conclusion

Analysis of the synthesis dynamics of IFN-α in patients with recurrent herpes simplex virus using etiotropic and immunotropic therapy demonstrated the following:

1. Synthesis of IFN-α level as a response to activation in case of recurrence of herpes simplex virus IFN-α increases, although with different intensity in saliva and blood depending on the severity of the disease.

2. Normalization of the IFN-α level in saliva and blood in different groups of patients after treatment and during follow–up, especially in the saliva of patients with recurrent HSV with a mild disease, and in blood – with a medium to severe and severe disease.

3. The use of inosine pranobex in patients with different severity of recurrent HSV 1/2 does not cause local and systemic interferon refractoriness within 12 weeks of treatment.

4. We have established the clinical efficacy of antiviral treatment of patients with recurrent HSV: with a mild disease after treatment, it was 73.5%, during follow–up – 85.8 %; in patients with a medium to severe and severe disease after treatment – 64.0 %, during follow–up – 70.4 %.

References

1. Belikova EA, Ivanova GF. Sovremennyye predstavleniya o herpeticheskoy infektsyi. Lekarstvennyy vestnik. 2015; 3(59): 22–27. [In Russian]

2. Vyhovska OV. Herpesvirusni infektsyi u ditey: klasyfikatsiya, klinichni formy, proyavy, sotsialno-medychni aspekty. Dutaychyy likar. 2016; 4(49): 41–51. [in Ukrainian]

3. Gyliyk OG, Bulat LM. Osoblyvosti diahnostyky dyfuznykh urazen hepatobiliarnoyi systemy pry herpesvirusniy infektsyi v ditey, shcho zumovlena Epstein-Barr virusom (izolyovana ta u formi mikst infektsyi). Zaporozhye Medical Journal. 2018; 20(4): 503–508. [in Ukrainian]

4. Homolyaka IV, Starosyla DB, Zarkova LD. Morfolohichni ta virusologichni kharakterystyky eksperymentalnoyi virusnoyi infektsyi, vyklykanoyi virusom prostogo herpesu 1 typu. Laboratorna diagnostyka. 2012; 2(60): 41–45. [in Ukrainian]

5. Isakov VA, Isakov DV. Patogenez i lecheniye sotsialno znachimykh virusnych urogenitalnykh infektsiy (gerpesa i papilomavirusnoy infektsii). Klinicheskaya farmakologiya i terapiya. 2014; 23(1): 6–13. [In Russian]

6. Mikhaev OG. Prostyy herpes: patogenez, klinika ta likuvannya. Zdorovya Ukrainy. 2015; 1: 25–35. [in Ukrainian]

7. Chopyak VV. Lektsiyi z klinichnoyi imunolohii ta alergologiyi dlya praktychnykh likariv. Lviv: Vydavnytstvo LNMU imeni D.Halytskoho. 2014: 124–148. [in Ukrainian]

8. Danastas K, Miranda-Saksena M, Cunningham AL. herpes simplex virus type 1 interactions with the interferon system. International Journal of Molecular Sciences. 2020; 21, 5150.

9. Hugh JF, Biswas S. Antiviral drug resistance and helicase–primase inhibitors of herpes simplex virus. Drug Resistance Updates. 2011; 14(1): 45–51.

10. Kwiatkowski A, Gallois J, Bilbault N. Herpes encephalitis during natalizumab treatment in multiple sclerosis. Mult Scler. 2012; 18(6): 909–911.

11. Noska A, Kyrillos R, Hansen G, Hirigoyen D, Williams DN. The role of antiviral therapy in immunocompromised patients with Herpes Simplex virus meningitis. Clinical Infectious Diseases. 2015; 60(2): 237–242.

12. You Y, Wang L, Li Y, Wang Q, Cao S., Tu Y. et al. Multicenter randomized study of inosine pranobex versus acyclovir in the treatment of recurrent herpes labialis and recurrent herpes genitalis in Chinese patients. Journal of Dermatology. 2015; 42: 1–6.

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