PHARMACEUTICAL COMPOSITION IN THE FORM OF A TABLET AND A METHOD FOR PRODUCING SAME

Field of the Invention

The invention relates to the field of medicine, the chemical-pharmaceutical industry and, in particular, to agents exhibiting antiviral and immunogenic activity. It relates to new glutaryl histamine compositions and methods for producing same. In particular, glutaryl histamine formulations are mixed with various excipients for producing solid glutaryl histamine formulation.

In some embodiments a solid formulation is a tableted form, in other embodiments this is a capsule.

An additional aspect of this invention includes a method for producing preparative forms of glutaril histamine. Other aspects of this invention include use of these compositions for treatment of a viral disease in a mammal in need of this, which includes introducing a therapeutically efficient quantity of a composition according to this invention to this mammal.

Prior Art

Influenza presents a most serious problems among viral diseases.

Influenza is one of the most dangerous infection from the epidemical point of view. For example, annually about 7 million cases of an influenza virus infection are registered among 142 million of the population, which is about 20% in the structure of infectious and parasitic diseases, and this is a significant indicator (??). One of the evolutionary abilities of the influenza virus is its high ability to mutate quickly due to the antigen variability. Influenza viruses are widespread both in human beings and in several types of animals, including

mammals and birds. Thus, influenza viruses of some animals, e.g., birds and pigs, are the most pathogenic.

Vaccinal prevention is the most widespread form of acting upon an influenza virus. Vaccinotherapy gives good results in morbidity prevention and/or reduction in disease intensity. And even these measures cannot stop mass disease outbreaks, especially in the cold period of the year.

Mortality in cases of several influenza forms is more than 50%, even with high-technology methods of treatment. In such cases a disease, as other influenza forms of other etiology, is characterized by acute onset and severe clinical course, i.e., a high (40°C and higher) temperature and prolonged fever with intoxication symptoms - headache, insomnia, pain feelings in muscles and joints, meningeal symptoms. One of unfavorable clinical courses is an instant form, i.e., a quickly developing hemorrhagic toxic pulmonary edema and fatal outcome caused by respiratory failure and cardiovascular collapse [Int. J. Tuberc. Lung Dis. // 2007 - V.11. - N7. - P.710-721].

During influenza therapy Amantadine and Remantadine have been widely used – preparations that exhibit antiviral activity against influenza virus strains [Am. J. Med. - 1997. - V.102(3A) - N17. - P.55-60], [Watts J. «Asian nations step up action to curb spread of avian influenza» // Lancet. - 2004. - V.363 - N9406. - P.373]. The antiviral preparation Remantadine is widespread in the Russian Federation. It is used for treatment and prophylaxis of an infection caused by influenza viruses of A type. This preparation has shown a pronounced therapeutic effect in respect of the influenza virus of the H3N2 sub-type. The mechanism of action upon the virus is connected with blocking the viral protein function within the membrane. The virus activity is inhibited at the stages of receptor-mediated endocytosis, decapsidation in the phagolysosome as well as assembly and blastogenesis of virus particles [RAMN Vestnik (Bulletin

of the Russia Academy of Medical Sciences // 1993. No. 3. - p. 10-15]. However, Remantadine, as a representative of the adamantane group, has certain limitations in use. If a dose is high, side effects in the CNS appear, in particular, spasmodic effects. It also acts unfavorably on liver and kidneys due to which it is unlikely to be used for patients having diseases of these organs.

At present, inhibitors of neuraminidase of influenza viruses A and B are widely used, such as oseltamivir phosphate and zanamivir, which, by connecting to a hydrophobic region of an active region of neuraminidase of an influenza virus, blocks the latter's ability to detach remains of sialic acid from the surface of an infected cell, thus suppressing passage of newly formed virions out of it.

Oseltamivir is the trade name of Tamiflu®, zanamivir is the trade name of Relenza®; they are considered to be efficient antivirus preparations [Cheung C.L., Rayner J.M., Smith G.J. et al. Distribution 20 of amantadine-resistant H5N1 avian influenza variants in Asia // J. Infect. Dis. - 2006. - Vol.193. - P. 1626-1629]. At present, cases of appearing resistance to oseltamivir and zanamivir are reported [Menno D. de Jong, Tran Tan Thanh, Truong Huu Khanh et al. Oseltamivir Resistance during Treatment of Influenza A (H5N1) Infection // N. Engi. J. Med. - 2005. - N353. - P.2667-2572], [Smee D.F., Wong M.H., Bailey K.W., et al. Activities of oseltamivir and ribavirin used alone and in combination against infections in mice with recent isolates of influenza A (H1N1) and B viruses // Antivir Chem Chemother. - 2006. - V.17, N4. - P.185-192]. It is related to mutations in the gene coding the M_2 protein, which mutations make the virus resistant to this class of compounds [Li K.S., Guan Y., Wang J., et al. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia // Nature. - 2004. - V.430, N6996. - P.209-213], [Hui-Ling Y., Ilyshina N.A., Salomon R. et al. Neuraminidase inhibitor-

resistant recombinant A/Vietnam/1203/04 (H5N1) influenza viruses retain their replication efficiency and pathogenicity in vitro and vivo // J. Virol. - 2007. - Vol.81. - P.12418-12426; Le Q.M., Kiso M., Someya K. et al. Avian flu: isolation of drug-resistant H5N1 virus // 30 Nature. - 2005. - Vol.437. - P.1108]. According to some data, mutations appear in about 30% of cases. Also, neuraminidase inhibitors exhibit pronounced side effects in respect of the CNS. Thus, experts of the British Health Protection Agency gave their recommendations to the British Government to the effect that oseltamivir, or Tamiflu, should not be administered to children for A/H1N1 influenza prophylaxis due to a great number of unwanted side effects.

One of the most common preparations for treatment and prophylaxis of viral infections, including influenza, in the Russian Federation is Arbidol®. However, if it is administered orally against highly pathogenic strain of influenza virus A (H5N1) in vivo according to regimens of urgent prophylaxis and treatment (135 mg/kg of white mice body weight), the efficiency of its protection is only 25 and 10%, respectively. This protection level does not comply with the existing national requirements to efficiency of antivirus preparations (at least 30%) [see, "Manual for Experimental (Pre-Clinical) Studies of New Pharmacological Substances". - M., 2005. - p. 541].

Highly pathogenic virus diseases also include the severe acute respiratory syndrome (SARS) that is a new acute coronaviral disease caused by the agent of genotype IV and characterized by mortality up to 10% [Revised U.S. Surveillance case definition for severe acute respiratory syndrome (SARS) and update on SARS cases - United States and worldwide, December 2003 // MMWR Wkly Rep. - 2003. - Vol.52, N.49. - P.1202- 1206].

Summary of the Invention

According to this invention the method for producing a glutaryl histamine composition that does not comprises magnesium salts of silicic acids and/or talc is proposed. Glutaryl histamine compositions comprising one or more diluents, disintegrators, binders and lubricants are proposed. One practical embodiment proposes a composition that comprises glutaryl histamine and one or more diluents, disintegrators, binders and lubricants.

Adjuvants are selected for the purpose of delivering a corresponding amount of glutaryl histamine in a formulation made industrially. All adjuvants are selected so as to be inert, organoleptically acceptable and compatible with glutaryl histamine. Adjuvants that are used in solid preparative forms for oral administration include, as a rule, excipients or diluents, binders, disintegrators, lubricants, anti-adhesive agents, sliding agents, wetting agents and surfactants, pigments, flavoring agents, sweeteners, adsorbents and agents improving organoleptic properties.

Excipients or diluents are added to an active substance in order to increase a tablet volume. They include lactose that may be either crystalline or amorphous. Various types of lactose include lactose monohydrate, α-lactose monohydrate, such as anhydrous forms of lactose (α-, β-) and agglomerated lactose. And one of the most common excipients is lactose monohydrate. Microcrystalline cellulose and cellulose microparticles are also used.

Many diluents also act as disintegrators and binders; and these additional properties have been taken into account while developing the formulation. Disintegrators are included into tableted formulations in order tablets may disintegrate into particles of an active pharmaceutical component and adjuvants, which would facilitate dissolution of an active component and improve its

bioavailability. The disintegrator is sodium cross-carmellose, microcrystalline cellulose.

Binders are used for wet granulation. The function of a binder is to make a powder loose and facilitate pressing. Binders are cellulose derivatives, such as microcrystalline cellulose, polyvinylpyrrolidone.

Lubricants are used in tableted preparative forms for preventing a tablet from adhering to an impact surface and for reducing friction at pressing steps. Lubricants include salts of stearic acid, preferably calcium stearate, and mineral salts, for example talc

Sliding agents are used in solid formulations for improving fluidity of a tablet mass. For this purpose microcrystalline cellulose; calcium stearate;talc are used.According to the embodiments, these compositions comprise, preferably, but not exclusively, one standard dose of glutaryl histamine. Preferably, the standard dose is in the form of a solid formulation, such as a tablet or a capsule, and, more preferably, is a tablet. In particular, this tablet may comprise 30, 60, 100 and, preferably, 90 mg of glutaryl histamine in a solid formulation of 250 mg.

The objective of the invention is to expand the range of agents exhibiting antiviral and immunogenic activity.

This objective is achieved by the new pharmaceutical composition in a solid drug formulation, which exhibits antiviral and immunogenic activity and is characterized in that it comprises, as the active substance, glutaryl histamine in an amount up to 75 weight % and adjuvants that form the rest. This formulation may comprise, as adjuvants, lactose monohydrate, microcrystalline cellulose, sodium cross-carmellose, colloid silicon dioxide (Aerosil 300) and calcium stearate. Such high content of the active substance is not disclosed in the state of the art.

The new pharmaceutical composition in the form of a solid formulation exhibiting antiviral and immunogenic activity is characterized in that it comprises glutaryl histamine as the active substance and lactose monohydrate, microcrystalline cellulose, sodium cross-carmellose, colloid silicon dioxide (Aerosil 300) and calcium stearate as adjuvants, the said ingredients having the following content, in weight per cent:

Glutaryl histamine	18.0-75
Microcrystalline cellulose	18.0-71
Sodium cross-carmellose	0.25-1
Colloid silicon dioxide (Aerosil 300)	0.5-2
Calcium stearate	0.5-2
Lactose monohydrate	the rest.

In order to further raise the composition stability, the active substance is first dried to a moisture content not more than 1.5%.

A single oral dose causes an increase in the blood IFN content within the physiological norm upper limits in 73.3% of donors in 24-48 hours, stimulates and normalizes reduced alpha-IFN-producing ability of leucocytes in practically healthy animals in 24 hours; a single intake of the glutaryl histamine composition stimulates the gamma-IFN production.

In order the claimed composition may achieve pharmacological activity, production process factors, such as a time period of mixing the ingredients, sequence of mixing them as well as the necessity of sifting the components in advance, have been identified.

According to an industrial embodiment of this invention, the method for producing the pharmaceutical composition in a tablet form is provided, said method comprising the following steps: sifted powder of microcrystalline cellulose is thoroughly mixed for 1-2 minutes, sifted powder of glutaryl

histamine and sifted lactose monohydrate are added; the obtained mixture is thoroughly mixed for 5-7 minutes; then, sifted powder of sodium cross-carmellose is added with mixing for 3-5 minutes; sifted powders of colloid silicon dioxide and calcium stearate are added with mixing continued for 2-4 minutes; the finally obtained mixture is tableted by direct pressing of the solid formulation of glutaryl histamine. The final mixture may be encapsulated.

Best Mode for Carrying Out the Invention

Example A.

Composition.

Active substance:	
glutaryl histamine	30.00 mg
Adjuvants:	
lactose monohydrate (BP/Eur.Ph. or USP-NF)	34.00 mg
microcrystalline cellulose (BP/Eur.Ph. or USP-NF)	47.62 mg
sodium cross-carmellose (BP/Eur.Ph. or USP-NF)	0.68 mg
Aerosil 300 (GOST 14922-77 or BP/Eur.Ph. or USP-NF)	1.35 mg
calcium stearate (BP/Eur.Ph. or USP-NF)	1.35 mg
Tablet weight	135.00 mg

Example B. Composition.

Active substance:	
glutaryl histamine	100.00 mg
Adjuvants:	

lactose monohydrate (BP/Eur.Ph. or USP-NF)	68.00 mg
microcrystalline cellulose (BP/Eur.Ph. or USP-NF)	95.25 mg
sodium cross-carmellose (BP/Eur.Ph. or USP-NF)	1.35 mg
colloid silicon dioxide (Aerosil) (GOST 14922-77 or BP/Eur.Ph. or USP-NF)	2.70 mg
calcium stearate (BP/Eur.Ph. or USP-NF)	2.70 mg
Tablet weight	270.00 mg

Tablets should have white color or white color with cream tint, the shape of flat cylinder with a bevel and a notch, unbroken edges, smooth and uniform surface and should not have cracks or cleavages.

Values of the pharmacokinetic parameters did not show any essential deviations during the study.

The elimination rate constant is 0.22±0.004.

The absorption rate constant is 1.695±0.028.

Time of reaching a maximum concentration, Tmax, hours, is 1.3±0.1.

The maximum concentration, Cmax, μg/mL, is 70.3±2.0

Example 2. Studies of the formulation.

3.1. Toxicological studies.

Table 1.

Kind of study	Types of animals	Method of introduction	Doses	Period of observation

Acute toxicity of the substance	Rats, mice	Orally, intraperitoneally	Up to 3,000 mg/kg	14 days
Chronic toxicity of the substance	Rats	Orally, intraperitoneally	2,5 and 25 mg/kg	6 months
	Dogs	Orally	2,5 mg/kg	3 months
Acute toxicity of the final formulation	Mice	Orally	150, 300, 450, 600 and 750 mg	14 days
Sub-acute toxicity of the final formulation	Rabbits	Orally	12 and 36 mg/kg	1 month

Table 2.

Kind of study	**Types of animals**	**Doses studied**	**Type of study**
Allergenicity	Cavies,	500, 5000 µg/kg	in vivo
	Non-linear mice (n=12)	11.5 mg/kg	in vivo
Reproductive toxicity	Rats of both genders	2.5 and 25 mg/kg	in vivo
Mutagenicity	Mice	12.5 mg/kg	in vivo
	Ames test	1.0, 10.0, 100, 1000, and 10000 µg /mL	in vitro
Carcinogenicity	Rats and mice	0.05, 0.5, 5.0 mg/kg	in vivo

The toxicological study results

1. According to data on acute and chronic toxicity, the glutaryl histamine composition may be referred to a relatively harmless preparation.

2. The composition does not exhibit allergenic and mutagenic properties.

3. The glutaryl histamine composition does not exhibit reproductive toxicity.

4. The composition in all the doses studied (0.05, 0.5, 5.0 mg/kg of glutaryl histamine) does not exhibit carcinogenic activity that was shown in the conditions of a 24-month experiment.

3.2. Study of the glutariyl histamine composition efficiency in relation to an A-influenza agent and adenoviruses in experiments in vitro and in vivo.

Materials and methods:

The following materials were used for the in vitro studies:

- A/Aichi/2/68 (H3N2) and A/hen/Kurgan/Russia/2/05 (H5N1) strains;

- GMK-AH-1 constant cultures of green-monkey kidney cells, MDCK dog kidneys;

- PS-4 growth semi-synthetic medium.

The following materials were used for the studies in vivo:

- white outbred mice having the weight of 15 g that are obtained from the vivarium of VTs NIIM, Moscow Region, Russia.

The laboratory animals were infected intranasally.

During the studies in vitro at the infective dose of 0.1 of a cytopathic dose 50/cell the glutaryl histamine composition in a concentration of 100-200 μg/mL efficiently suppresses the reproduction of the influenza virus A (strain A/Aichi/2/68 (H3N2) и A/hen/Kurgan/Russia/2/05 (H5N1)) when delivered in 2 hours after infecting a mono-layer.

During the studies in vivo the glutaryl histamine composition at the dose of 5 mg/kg is efficient as a prophylactic drug in relation to the influenza A (H3N2 and H5N1).

The prophylactic efficiency of the glutaryl histamine composition is slightly inferior in comparison to Tamiflu.

When used according to the urgent prophylaxis system and for treatment, the glutaryl histamine composition is inferior in comparison with Tamiflu. The optimal dose of the glutaryl histamine composition according to the said system is 15 mg/kg.

3.3. A study of interferon-inducing and immunomodulating activity of the glutaryl histamine composition:

- preparation and doses: glutaryl histamine composition, oral introduction;

- material for the study: blood samples of 15 healthy rats before and after introduction of the preparation;

materials and methods:

indices of the IFN-status were monitored in 2, 4, 6, 8, 12, 24, 48 hours after the introduction of the preparation.

3.3.1. The results of the study of interferon-inducing and immunomodulating activity of the glutaryl histamine composition:

single oral introduction raises the interferon content by 73.3% of the donors within the upper limits of the physiological norm in 24-48 hours;

the composition stimulates and normalizes a lowered α-IFN-producing ability of leucocytes in practically healthy animals in 24 hours;

single introduction of the glutaryl histamine composition stimulates the γ-IFN production.

Thus, the new composition has showed high results of pharmacological activity and may be recommended for the introduction into the clinical practice.

Industrial Applicability

The invention is applicable in the field of medicine, the chemical-pharmaceutical industry, namely for the production of agents exhibiting antiviral and immunogenic activity, and relates to new glutaryl histamine compositions and methods for making same.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition in a solid formulation exhibiting antiviral and immunogenic activity, characterized in that it comprises glutaryl histamine as the active substance, lactose monohydrate, microcrystalline cellulose, sodium cross-carmellose, colloid silicon dioxide and calcium stearate as adjuvants, the ingredients having the following contents, in weight per cent:

glutaryl histamine	18.0-75.0
microcrystalline cellulose	18.0-71.0
sodium cross-carmellose	0.25-1.0
colloid silicon dioxide	0.5-2.0
calcium stearate	0.5-2.0
lactose monohydrate	the rest.

2. The pharmaceutical composition according to Claim 1, characterized in that the active substance is first dried to moisture content not more than 1.5%.

3. The pharmaceutical composition according to Claim 1, characterized in that the glutaryl histamine composition is used in the form of a capsule.