

Description

This invention relates to pharmaceutical compositions, and more particularly to pharmaceutical compositions useful for treatment of viral infections such as herpes infections in mammals.

Herpes simplex virus (HSV) infections are widespread in human populations, and pose a particularly difficult health problem. Genital herpes poses a serious health threat to women, in particular. Pregnant women with active genital herpes infections at the time of delivery have a 50—50 chance of passing it on to their babies. The American Academy of Pediatrics states that 60% of those babies born with HSV infections will die, and half of the survivors will suffer severe damage to the brain, nervous system and eyes ("Pediatrics" 66, 147—9, 1980). It has also been proposed that HSV-2 may have a role in the onset of cervical cancer. There has been observed an association between sexual intercourse and cervical cancer, which may be explained by transmission of HSV-2.

Unlike other sexually transmitted diseases such as gonorrhea, syphilis and nongonococcal urethritis, there is currently no cure for herpes infections. Many of the drugs currently in clinical use may not be effective in reducing the severity or the duration of the symptoms. Even after the symptoms disappear, herpes virus tends to remain dormant in nerve tissue, only to be reactivated at a later date to an active phase of infection, causing lesions ("cold sores") and other symptoms to recur. A drug can be considered effective if it diminishes the severity of the lesions, allows for more rapid healing, extends the period between the recurrences of herpes infections or stops recurrences altogether.

Herpes simplex virus is one member of the family "Herpetoviridae"; other members of this family which infect humans are varicella-zoster, cytomegalovirus and Epstein-Barr virus. The family also includes various members which attack animals. For example, there are three types of equine herpesvirus, a swine herpesvirus, a canine herpesvirus and a feline herpesvirus, among others.

As with all viruses, herpes virus invades healthy host cells on which it relies to provide its needs for replication. Herpes viruses code for some of the enzymes they need for replication, instead of relying completely on the host cell for all their needs. Hence, herpes viruses are subject to selective inhibition by certain drugs that interfere specifically with viral enzymes.

Our WO 83/02723 describes mixtures of 9 - [[2 - hydroxy - 1 - (hydroxymethyl) - ethoxy]methyl]guanine and PFA or PAA or salts thereof, for use in treatment of herpes virus infected cells.

It has now been found that therapeutic compositions having good activity against herpes virus can be prepared from combinations of interferon and 9 - [[2 - hydroxy - 1 - (hydroxymethyl) - ethoxy]methyl]guanine, or a pharmaceutically acceptable salt thereof. These combinations are much more effective than one could have predicted from a consideration of the activities of the compounds individually, indicating that some form of synergic effect is taking place.

According to the present invention, a pharmaceutical composition which can have activity against herpes virus infections, comprises, in combination, an interferon compound and 9 - [[2 - hydroxy - 1 - (hydroxymethyl)ethoxy]methyl]guanine or a pharmaceutically acceptable salt thereof.

The invention envisages the administration of the two active compounds, separately or sequentially (in any order). For use in these or simultaneous administrations, the compounds may be formulated independently, e.g. in one preparation or package. The invention will be discussed, by way of example, in terms of a composition as described above.

Interferon is a low molecular weight polypeptide produced as an excretion from various types of viable mammalian cells. Its properties, chemical nature and methods of preparation and recovery have been extensively studied and documented in recent years, because of its potentially valuable pharmacological properties. It is known to exist in several types. Whilst any type of interferon may be useful in a composition of the present invention, it is preferred to use interferon- γ or interferon- α or a mixture thereof. Interferon concentrations are commonly expressed as standard "units" which are internationally accepted and documented, and relate to the potency of a given quantity of interferon to inhibit a virus replication under standard conditions.

9 - [[2 - Hydroxy - 1 - (hydroxymethyl)ethoxy]methyl]guanine (hereinafter sometimes referred to as G*) and processes for its preparation are described in EP—A—0072027, EP—A—0074306, US—A—4355032 and our EP—A—0049072.

The compositions of the invention may of course include more than two active ingredients. G* may be present as the free base, or alternatively as a pharmaceutically acceptable salt such as hydrochloride, sodium salt, phosphate, diphosphate etc.

The preferred compositions of the present invention appear to be active against a wide representative variety of strains of HSV, both types I and II. The compositions may also be active against equine herpesvirus of various types, and swine herpesvirus (pseudorabies virus).

Nearly every HSV strain produces virus particles which are partially resistant to each of the drugs mentioned above. For example, if one examines a typical titration curve which shows the effect of varying drug concentrations upon HSV plaque formations (the viral plaque titration method of Roizman and Roane referred to in more detail below), the curve is sigmoid, i.e. there are a few viral plaques which emerge in the presence of drug concentration which readily suppresses other plaques. It is these partially drug-resistant virus plaques which can sometimes be suppressed by a second drug possibly having a mode of action

different from the first drug. The result may be synergistic action between the first and second drugs, as set out below.

The relative amounts of the drugs in the compositions according to the invention can be varied over wide limits. The optimum amount of each drug varies according to the nature of the formulation in which the drugs are to be applied, the type and strain of HSV to be treated, and the severity and location of the infection, among other factors. The amount of interferon, as noted above, is commonly expressed in standard units. Generally, compositions containing from about 50—500,000 units interferon per microgram of G* are effective. Preferred compositions are those containing from about 1,000—200,000 units of interferon per microgram of G*.

For administration to patients, the compositions of the invention may be applied topically as ointment, cream or powder, parenterally, interthecally, as nose drops, eye drops or as an aerosol for inhalation, again depending upon the nature and location of the infection to be treated. Effective unit doses for administration of the compositions interthecally or parenterally are suitably in the range from about 0.1—100 mg of total drugs in the chosen combinations, per kg mammal body weight, most suitably in the 0.5—20 mg per kg and most preferably about 5 mg per kg, on the basis of a dosage administered from 2—4 times daily. It is preferred to treat the infection with relatively large doses of the combination of drugs at the outset, so as to limit the chances of development of resistant viral strains in the infection.

For topical administration, ointments or creams in conventional inert bases (e.g. petrolatum) can be formulated, in the known way. An amount from about 0.1—10 weight per cent of total drugs, preferably from about 0.5—5 weight per cent of total drugs, provides a suitable concentration in an ointment or cream, for topical administration 1—4 times per day. Such topically applied formulations are effectively holding a reservoir of the active drugs against the infected site, so that the concentrations of drugs in the formulations are not critical, provided of course that a dosage level harmful to surrounding skin areas is not used.

The invention is further illustrated in the following specific experimental results and examples.

Example 1

Human fetal fibroblasts (HFF) derived from fetal tissues were used in these experiments. Cells were grown and maintained in Basal Medium Eagle (BME) supplemented with 0.112% sodium bicarbonate, 2 mM glutamine, 2 mg% Neomycin and 20% (vol/vol) calf serum.

HSV-1-Patton strains, an old-established, well-known strain of herpes simplex type 1 virus was used in the tests, as set out in the Tables presented below.

A viral plaque titration method (Roizman and Roane, 1961) was used to determine the titer of the HSV strain. Tissue culture dishes (35 by 10 mm, Corning) were seeded with cells and used for assays when they were approximately 75% monolayer. Volumes (0.2 ml) of logarithmic dilutions of the virus strain were inoculated onto each of two tissue culture dishes and adsorbed for 1 hr with intermittent shaking, the inoculum was removed and 2 ml of 20% BME containing 0.5% human immune serum globulin was added. After a 48 hr. incubation period at 36°C in a 5% CO₂ atmosphere, the overlay medium was removed, and the cell sheets were stained with a 0.05% aqueous crystal violet solution. The plaque numbers were counted with the aid of a Nikon profile projector which magnified the dishes 10x. Duplicate results were averaged, and the number of plaque-forming units (PFU) was calculated. The virus titre is thus expressed as a number of plaque forming units to be seen after growth under these conditions.

As antiviral drugs in these experiments, there were used combinations of G* and interferon-γ.

In order to make comparisons between the activities of the various drugs and combinations, experiments were conducted by plaque titration to observe the antiviral potency, by observing and counting the number of PFUs per disc after growth of the infected cells in culture medium. For this purpose, tissue culture dishes (35 by 10 mm) with HFF cell monolayers at 75% confluence were inoculated with approximately 50 plaque-forming units of virus per 0.2 ml, and the virus was allowed to adsorb for 1 hr with intermittent shaking. After removal of the inoculum, 2 ml of 20% BME with 0.5% immune globulin and threefold dilutions of the appropriate drug were added to duplicate dishes. One set of dishes received no drug. After a 48 hr incubation period at 36°C in a 5% CO₂ atmosphere, the overlay medium was removed the cells were stained as described above, and plaques were counted. The counts of duplicate plates were averaged.

Combinations of the drugs were tested for activity by viral plaque titration, at various concentrations, and the drugs were individually plaque titrated for comparison purposes.

The results are given in the following Table. The virus titre results are expressed in plaque-forming units (PFU) per dish and, of course, the lower the figure, the greater the inhibitory, anti-viral effect of the tested drug.

TABLE 1
Plaque inhibition tests against HSV-1-Patton-infected HFF cells (PFU/dish)

G* (μg/ml)	Interferon (units/ml)			
	10	100	1000	0
0.02	27	23	24	31
0.064	24	26	19	29
0.2	18	20	7	18
0.64	1	0	0	1
2.0	0	0	0	0
0	30	24	24	36

These figures show that, whilst interferon-γ alone has only slight effect on the virally-infected cells and G* has effect at relatively high concentration, the combination in suitable proportions is much more effective, and permits the amount of compound G* to be reduced very significantly.

Example 2

The procedures of Example 1 were essentially repeated, but using mixtures of equal proportion of interferon-γ and interferon-α, with various amounts of G*, and again using HFF cells infected with HSV-1-Patton. In each experiment, the amount of interferon was 1000 units per ml of interferon-γ and 1000 units per ml of interferon-α, for a total amount of 2000 units per ml of interferon. The virus titre results, in this case, are expressed as plaque-forming units per ml, $\times 10^5$. The figure for "Fold Titre Reduction" is the ratio of the virus titre for the control, i.e. no drug, to that of the drug-present experiment. The results are presented in Table 2 below.

TABLE 2

G* Concentration micrograms/ml reduction	With interferon		No interferon	
	PFU/ml $\times 10^5$	Fold titre reduction	PFU/ml $\times 10^5$	Fold titre
0.02	30	4.0	115	1.0
0.064	5	24.0	90	1.3
0	100	1.2	120	1.0

The results show that synergistic action occurs in these combinations of drugs, against the tested HSV strains. At least the same degree of synergy is to be expected in vivo.

Example 3

In a further experiment, similarly virally infected HFF cells were incubated as described, and plaque titrated with various combinations of G* and an interferon-γ/interferon-α combination. In one set of dishes, no G* was used, and in the other set of dishes 0.05 micrograms per ml of G* was used. The interferon was in each case a mixture of equal amounts of interferon-γ and interferon-α, the numbers shown in the following results being the individual amounts of each component in the mixture.

The results are shown in Table 3. The figures are presented as "fold titre reduction", with the dishes containing no drugs being assigned the value of unity.

TABLE 3
(Fold titre reduction)

G* (µg/ml)	Interferon (units/ml)			
	0	10	100	1000
0	1.0	1.1	1.1	1.6
0.05	1.0	1.3	3.0	27

The relative amounts and concentrations of drugs used in these examples are appropriate for in vitro testing and to demonstrate the synergy of the combinations, but are illustrative only. Relative amounts in combinations for in vivo use and in practical administration for HSV treatments are as discussed previously, and may not bear close relationship to the proportions shown in the specific examples herein.

Claims for the Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A pharmaceutical composition which comprises, in combination, an interferon compound and 9 - [[2 - hydroxy - 1 - (hydroxymethyl)ethoxy)methyl]guanine or a pharmaceutically-acceptable salt thereof.
2. A composition which comprises an interferon compound and 9 - [[2 - hydroxy - 1 - (hydroxymethyl)ethoxy)methyl]guanine or a pharmaceutically-acceptable salt thereof, in a combined formulation, for simultaneous, separate or sequential use in therapy.
3. A composition according to any preceding claim, which comprises from 50 to 500,000 units of the interferon compound per microgram of the guanine compound or its salt.
4. A composition according to any preceding claim, in which the interferon compound is interferon-γ.
5. A composition according to any of claims 1 to 3, in which the interferon compound is interferon-α.
6. A composition according to any of claims 1 to 3, in which the interferon compound comprises a mixture of interferon-γ and interferon-α.
7. A composition according to any of claims 4 to 6, which comprises from 1000 to 200,000 units of the interferon compound per microgram of the guanine compound or its salt.
8. A composition according to any preceding claim, for use in the treatment of herpes virus infections in mammals.

Claims for the Contracting State: AT

1. A process for preparing a pharmaceutical composition, which comprises combining 9 - [[2 - hydroxy - 1 - (hydroxymethyl)ethoxy)methyl]guanine or a pharmaceutically-acceptable salt thereof and, per microgram of that compound or salt, from 50 to 500,000 units of an interferon compound.
2. A process according to claim 1, wherein the interferon compound is interferon-γ.
3. A process according to claim 1, wherein the interferon compound is interferon-α.
4. A process according to claim 1, wherein the interferon compound comprises a mixture of interferon-γ and interferon-α.
5. A process according to any preceding claim, in which from 1,000 to 200,000 units of the interferon compound are used per microgram of the guanine compound or its salt.

Patentansprüche für die Vertragsstaaten: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Pharmazeutische Zusammensetzung, die eine Interferonverbindung und 9 - [[2 - Hydroxy - 1 - (hydroxymethyl) - äthoxy] - methyl] - guanin oder ein pharmazeutisch annehmbares Salz hiervon in Kombination umfaßt.
2. Zusammensetzung, die eine Interferonverbindung und 9 - [[2 - Hydroxy - 1 - (hydroxymethyl) - äthoxy] - methyl] - guanin oder ein pharmazeutisch annehmbares Salz hiervon in einer kombinierten Formulierung für gleichzeitige, getrennte oder aufeinanderfolgende Verwendung in der Therapie umfaßt.
3. Zusammensetzung nach einem der vorhergehenden Ansprüche, die 50 bis 500 000 Einheiten der Interferonverbindung pro Mikrogramm der Guaninverbindung oder ihres Salzes umfaßt.
4. Zusammensetzung nach einem der vorhergehenden Ansprüche, in der die Interferonverbindung Interferon-γ ist.
5. Zusammensetzung nach einem der Ansprüche 1 bis 3, in der die Interferonverbindung Interferon-α ist.
6. Zusammensetzung nach einem der Ansprüche 1 bis 3, in der die Interferonverbindung eine Mischung von Interferon-γ und Interferon-α ist.
7. Zusammensetzung nach einem der Ansprüche 4 bis 6, die 1000 bis 200 000 Einheiten der Interferonverbindung pro Mikrogramm der Guaninverbindung oder ihres Salzes umfaßt.

TABLE 3
(Fold titre reduction)

G* (µg/ml)	Interferon (units/ml)			
	0	10	100	1000
0	1.0	1.1	1.1	1.6
0.05	1.0	1.3	3.0	27

The relative amounts and concentrations of drugs used in these examples are appropriate for in vitro testing and to demonstrate the synergy of the combinations, but are illustrative only. Relative amounts in combinations for in vivo use and in practical administration for HSV treatments are as discussed previously, and may not bear close relationship to the proportions shown in the specific examples herein.

Claims for the Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A pharmaceutical composition which comprises, in combination, an interferon compound and 9 - [[2 - hydroxy - 1 - (hydroxymethyl)ethoxy]methyl]guanine or a pharmaceutically-acceptable salt thereof.
2. A composition which comprises an interferon compound and 9 - [[2 - hydroxy - 1 - (hydroxymethyl)ethoxy]methyl]guanine or a pharmaceutically-acceptable salt thereof, in a combined formulation, for simultaneous, separate or sequential use in therapy.
3. A composition according to any preceding claim, which comprises from 50 to 500,000 units of the interferon compound per microgram of the guanine compound or its salt.
4. A composition according to any preceding claim, in which the interferon compound is interferon-γ.
5. A composition according to any of claims 1 to 3, in which the interferon compound is interferon-α.
6. A composition according to any of claims 1 to 3, in which the interferon compound comprises a mixture of interferon-γ and interferon-α.
7. A composition according to any of claims 4 to 6, which comprises from 1000 to 200,000 units of the interferon compound per microgram of the guanine compound or its salt.
8. A composition according to any preceding claim, for use in the treatment of herpes virus infections in mammals.

Claims for the Contracting State: AT

1. A process for preparing a pharmaceutical composition, which comprises combining 9 - [[2 - hydroxy - 1 - (hydroxymethyl)ethoxy]methyl]guanine or a pharmaceutically-acceptable salt thereof and, per microgram of that compound or salt, from 50 to 500,000 units of an interferon compound.
2. A process according to claim 1, wherein the interferon compound is interferon-γ.
3. A process according to claim 1, wherein the interferon compound is interferon-α.
4. A process according to claim 1, wherein the interferon compound comprises a mixture of interferon-γ and interferon-α.
5. A process according to any preceding claim, in which from 1,000 to 200,000 units of the interferon compound are used per microgram of the guanine compound or its salt.

Patentansprüche für die Vertragsstaaten: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Pharmazeutische Zusammensetzung, die eine Interferonverbindung und 9 - [[2 - Hydroxy - 1 - (hydroxymethyl) - äthoxy] - methyl] - guanin oder ein pharmazeutisch annehmbares Salz hiervon in Kombination umfaßt.
2. Zusammensetzung, die eine Interferonverbindung und 9 - [[2 - Hydroxy - 1 - (hydroxymethyl) - äthoxy] - methyl] - guanin oder ein pharmazeutisch annehmbares Salz hiervon in einer kombinierten Formulierung für gleichzeitige, getrennte oder aufeinanderfolgende Verwendung in der Therapie umfaßt.
3. Zusammensetzung nach einem der vorhergehenden Ansprüche, die 50 bis 500 000 Einheiten der Interferonverbindung pro Mikrogramm der Guaninverbindung oder ihres Salzes umfaßt.
4. Zusammensetzung nach einem der vorhergehenden Ansprüche, in der die Interferonverbindung Interferon-γ ist.
5. Zusammensetzung nach einem der Ansprüche 1 bis 3, in der die Interferonverbindung Interferon-α ist.
6. Zusammensetzung nach einem der Ansprüche 1 bis 3, in der die Interferonverbindung eine Mischung von Interferon-γ und Interferon-α ist.
7. Zusammensetzung nach einem der Ansprüche 4 bis 6, die 1000 bis 200 000 Einheiten der Interferonverbindung pro Mikrogramm der Guaninverbindung oder ihres Salzes umfaßt.



8. Zusammensetzung nach einem der vorhergehenden Ansprüche zur Verwendung bei der Behandlung von Herpesvirusinfektionen in Säugern.

Patentansprüche für den Vertragsstaat: AT

- 5 1. Verfahren zur Herstellung einer pharmazeutischen Zusammensetzung, welches das Vereinigen von 9 - [[2 - Hydroxy - 1 - (hydroxymethyl) - äthoxy] - methyl] - guanin oder eines pharmazeutisch annehmbaren Salzes hiervon und, pro Mikrogramm dieser Verbindung oder des Salzes, 50 bis 500 000 Einheiten einer Interferonverbindung umfaßt.
- 10 2. Verfahren nach Anspruch 1, worin die Interferonverbindung Interferon- γ ist.
3. Verfahren nach Anspruch 1, worin die Interferonverbindung Interferon- α ist.
4. Verfahren nach Anspruch 1, worin die Interferonverbindung eine Mischung von Interferon- γ und Interferon- α umfaßt.
5. Verfahren nach einem der vorhergehenden Ansprüche, worin 1000 bis 200 000 Einheiten
- 15 Interferonverbindung pro Mikrogramm der Guaninverbindung oder ihres Salzes verwendet werden.

Revendications pour les Etats Contractants: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Une composition thérapeutique qui comprend, en combinaison, un composé interféron et la 9 - [[2 - hydroxy - 1 - (hydroxyméthyl)éthoxy]méthyl]guanine ou un de ses sels acceptables en pharmacie.
- 20 2. Une composition qui comprend un composé interféron et la 9 - [[2 - hydroxy - 1 - (hydroxyméthyl)éthoxy]méthyl]guanine ou un de ses sels acceptables en pharmacie, dans une formulation combinée pour l'utilisation simultanée, séparée ou successive en thérapie.
3. Une composition selon l'une quelconque des revendications précédentes, qui comprend de 50 à
- 25 500 000 unités du composé interféron par microgramme du composé guanine ou de son sel.
4. Une composition selon l'une quelconque des revendications précédentes, dans laquelle le composé interféron est l'interféron γ .
5. Une composition selon l'une quelconque des revendications 1 à 3, dans laquelle le composé interféron est l'interféron α .
- 30 6. Une composition selon l'une quelconque des revendications 1 à 3, dans laquelle le composé interféron comprend un mélange d'interféron γ et d'interféron α .
7. Une composition selon l'une quelconque des revendications 4 à 6, qui comprend de 1 000 à 200 000 unités du composé interféron par μg du composé guanine ou de son sel.
8. Une composition selon l'une quelconque des revendications précédentes, pour l'utilisation dans le
- 35 traitement des infections à virus d'herpès chez les mammifères.

Revendications pour l'Etat Contractant: AT

1. Procédé pour la fabrication d'une composition pharmaceutique, qui consiste à combiner la 9 - [[2 -
- 40 hydroxy - 1 - (hydroxyméthyl)éthoxy]méthyl]guanine ou un de ses sels acceptables en pharmacie avec 50 à 500 000 unités d'un composé interféron par μg du composé guanine ou de son sel.
2. Un procédé selon la revendication 1, dans lequel le composé interféron est l'interféron γ .
3. Un procédé selon la revendication 1, dans lequel le composé interféron est l'interféron α .
4. Un procédé selon la revendication 1, dans lequel le composé interféron comprend un mélange
- 45 d'interféron γ et d'interféron α .
5. Un procédé selon une quelconque des revendications précédentes dans lequel on utilise de 1 000 à 200 000 unités du composé interféron par μg du composé guanine ou de son sel.

