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(54) AGENT THERAPEUTIQUE CONTRE LE RHUME,  
CONTENANT DE L’ISOMALT COMME PRINCIPE ACTIF  
(54) THERAPEUTIC ANTI-COLD AGENT CONTAINING ISOMALT  
AS AN ACTIVE INGREDIENT  

(57) L’invention concerne l’utilisation de mélanges d’itols comme principes actifs thérapeutiques.  
(57) The invention relates to the use of sugar alcohol mixtures as therapeutic active substances.
Title: THERAPEUTIC ANTI-COLD AGENT CONTAINING ISOMALT AS AN ACTIVE INGREDIENT

Bezeichnung: ISOMALT ALS WIRKSTOFF ENTHALTENDES ERKÄLTUNGSMITTEL

Abstract

The invention relates to the use of sugar alcohol mixtures as therapeutic active substances.

Zusammenfassung

Die vorliegende Erfindung betrifft die Verwendung von Zuckeralkoholgemischen als therapeutische Wirkstoffe.
Therapeutic Anti-Cold Agent Containing Isomalt as an Active Ingredient

Description

This invention concerns the use of a sugar-alcohol mixture, containing 6-0- D-glucopyranosyl-D-sorbitol-(1,6-GPS) and 1-0- D-glucopyranosyl-D-mannitol-(1,1-GPM), in drugs, food and stimulants as a therapeutic, in particular immuno-stimulating and anti-bacterial, active ingredient, particularly in conjunction with zinc, and also concerns the products containing these substances.

An illness causing widespread and unpleasant symptoms is the common cold. Cold-causing microorganisms, for example bacteria like Staphylococcus aureus or viruses such as rhinoviruses, stay in the affected organism mainly in the neck, throat and nose areas, where they can generally be controlled directly with active substances. Suitable forms of presentation for pharmacologically-active substances are for instance hard pastilles, chewing gums or compressed lozenges. Such presentation forms are characterised in that they are solid formulations which are dissolved slowly in the neck and throat areas and in that the microorganisms, which are located superficially at or on the mucous membranes, are hindered in their multiplying and spreading.

Zinc is known as a pharmaceutically-effective substance for controlling microorganisms which cause colds. In a study of rhinoviruses, it was able to be shown that the antiviral effects depend directly on the amount of the uncombined Zn^{2+} ions (Merluzzi et al., in: Research Communications in Chemical Pathology and Pharmacology Vol. 66, (1989) 3, 425-440). Free, i.e. non-complex, zinc ions are regarded today as a pharmacological agent which markedly lessens the symptoms of cold illnesses both in their duration and severity (Mossed et al., in: Annals of Internal Medicine, Vol. 125 (1996) 2, 81-87 and Godfrey et al., in: Alternative Therapies, Vol. 2, (1996) 6, 63-72). An essential prerequisite for the observed therapeutic effect of zinc is the necessity of taking the pharmaceutical carrier containing the zinc ions, for example a hard pastille, as continuously as possible at regular intervals from the start of the appearance of the symptoms, for instance every 1.5 to 2 hours, and allowing it to dissolve in the mouth. The reason for this being that the zinc ions act topically in the mouth and nose/throat areas.

However, this type of continuous application of active ingredient in the mouth area brings with it a number of serious disadvantages, since the mono- and disaccharides available in by far the most instances in the pharmaceutical carrier promote the formation of caries. For this reason it is advantageous to use dental-friendly sugar-substitutes as a replacement for the caries-forming sugar. Sorbitol and mannitol are known as a sugar-substitute for use in preparations containing zinc. However, the use of sorbitol and mannitol brings with it the drawback that these dissolve quickly in the mouth and throat areas, so that the reaction time of the zinc ions, which is required to be as long and continuous as possible, is not always guaranteed. Furthermore, it is known from US
patents 5,409,905 and 5,002,970 for example, that sorbitol and mannitol have metal-complexing properties and also form complexes with zinc. From Godfrey et al., in: The Journal of International Medical Research 20 (1992), 234-246 and Zarembo et al., in: Journal of Pharmaceutical Sciences, Vol. 81, (1992) 2, 128-130, it is also known that mannitol and sorbitol are zinc-complexing in effect and in addition, that mannitol-, sorbitol- and zinc-containing hard pastilles have correspondingly markedly reduced effectiveness with regard to the temporal reduction and lessening of the cold symptoms (Smith et al., in: Antimicrobial Agents and Chemotherapy 33 (1989) 5, (646-648). The mannitol- and sorbitol-zinc complexes formed convert the zinc into a pharmaceutically inert form.

The underlying technical problem of this invention therefore consists in preparing a product which promotes a general stimulation of the immune defence and alleviates and controls cold illnesses, but at the same time is acarogenic and of reduced calorie content as well as ensuring a constantly efficient release of the active ingredient.

This invention solves this problem by making available in drugs, foods and stimulants the application of a sugar alcohol mixture containing 1,6-GPS and 1,1-GPM as a therapeutic active ingredient, in particular as an immuno-stimulant and/or as an antimicrobial agent. Surprisingly it was able to be shown that a mixture of 1,6-GPS and 1,1-GPM (in particular an approximately equimolar mixture of these two sugar alcohols containing 43-57 % by weight 1,1-GPM and 57-43 % by weight 1,6-GPS (based on DS, i.e dry substance), which is also described as isomalt, hydrated isomaltulose or Palatinol®) acts in an antimicrobial manner, but in addition also in a reduced calorie content and is acarogenic, has very good working properties as well as going into solution more slowly than sorbitol, so that an extended release of active ingredient is made possible. In a surprising way it was also proved that such a mixture of 1,6-GPS and 1,1-GPM has an immuno-stimulating effect and raises the immunity of the body and cell-specific immunity and resistance against infections.

In an especially advantageous form of implementing this invention, the sugar alcohol mixture containing 1,6-GPS and 1,1-GPM also has 1,1-GPS (1-O- D-glucopyranosyl-D-sorbitol) and if necessary small quantities of hydrated or unhydrated mono-, di- and oligosaccharides, xylitol, erythritol, maltitol, hydrated glucose and starch syrups, lactitol and polydextrose. Sugar alcohol mixtures usable according to the invention are known for example from EP 0 625 578 B1, which is included too in the disclosure content of this invention, with regard to such sugar alcohol mixtures and their preparation. In particular the invention concerns the above-mentioned sugar alcohol mixtures, in which these mixtures can contain for example 10-50 % by weight 1,6-GPS, 0.5-20 % by weight 1,1-GPS and 30-70 % by weight 1,1-GPM (based on DS). In a further preferred form of implementation, the mentioned sugar alcohol mixtures contain 5-10 % by weight 1,6-GPS, 30-40 % by weight 1,1-GPS and 45-60 % by weight 1,1-GPM (based on DS).
In the context of this invention, a drug is understood to mean an agent stimulating or restoring mainly the health of a human or animal body, which agent can be both prophylactic and healing in nature and includes a therapeutic active ingredient giving rise to this effect. A therapeutic active ingredient in the context of this invention therefore can promote curing of illnesses and disorders of the bodily functions as well as the prophylaxis.

In the context of this invention, a food is understood to mean a product, for example yoghurt, marmalade, including the so-called "functional foods", promoting mainly the nourishment of the human or animal body, whereas a stimulant is understood to mean a product, for example chewing gum, candy, caramel, chocolate, bread, cake and pastry, biscuit, jelly bear or similar. Both the foods and stimulants mentioned can be also described as drugs by virtue of their content of therapeutically active substances, that is 1,1-GPM and 1,5-GPS, preferably in combination with zinc.

As mentioned, the mixture of the sugar alcohols, particularly together with zinc, used according to the invention acts as a antimicrobial agent, or as an active ingredient mixture, that is to say the mixture has the following defined properties of an antimicrobial agent. In the context of this invention, an antimicrobial agent is understood to mean an active ingredient which acts on bacteria, in fact both gram-positive and gram-negative bacteria, protozoa, viruses, bacteriophages, retroviruses, viroids, yeasts, algae, fungi and/or similar microorganisms, in which the action consists in inhibiting the development and/or multiplication, and/or destruction of the microorganism, and therefore is microbistatic and/or microbicidal. In an especially preferred implementation form of the invention, an antimicrobial agent has in particular a bacteriostatic and/or bactericidal and/or antiviral action. This action preferably is developed particularly by using the active ingredient according to the invention in hard pastilles topically in the mouth and throat areas.

The mixture of the sugar alcohols used according to the invention, particularly together with zinc, has an immuno-stimulating effect, i.e. it improves in particular the body's own defensive powers against influences, both normal and foreign to the body, in particular parasites or bacterial or viral infections by pathogenic germs, including pathogens of the respiratory and gastrointestinal tract, and also acts against tumour formation. The immuno-stimulating action is distinguished in particular by the supply and diffusion of immuno-reactive substances. This action preferably develops because of a product according to the invention which is swallowed, i.e. is in the gastrointestinal tract.

In a particularly preferred implementation form of this invention, provision is made for combining the sugar alcohol mixtures, which contain 1,6-GPS and 1,1-GPM and are used according to the invention, with zinc, especially inorganic or organic zinc salts such as zinc gluconate or zinc acetate. Surprisingly it has been shown that although - as known from the
literature - sorbitol and mannitol form complexes with zinc and from there, sorbitol- or mannitol-containing products with a zinc content have no, or no substantial, cold-controlling or immuno-stimulating function, the sugar alcohol mixtures, which contain dissolved 1,6-GPS and 1,1-GPM and are used according to the invention and which contain the added zinc, show a similar complexing behaviour which is observed also in products based on saccharose/glucose mixtures, and therefore the zinc ions mostly exist in the non-complex form and accordingly can develop their antimicrobial and immuno-stimulating function. Among other things, this finding can therefore be regarded as surprising, that is to say that the disaccharide alcohols 1,6-GPS and 1,1-GPM are sorbitol or mannitol derivatives which ought to have formed complexes with the zinc as expected to a greater extent. The invention accordingly also concerns the use of a mixture of the aforementioned sugar alcohols and zinc ions as an antimicrobial agent, or that is as an antimicrobial combination of agents, especially as a therapeutic anti-cold preparation as well as an immuno-stimulant.

In the context of this invention, free or non-complex zinc ions are understood to mean zinc ions of the type which form only labile aquo complexes in aqueous solution, in which it has each ion in an aqueous medium in the form of a solvation sphere. Free or non-complex zinc ions are correspondingly not associated with other substances in a form of a complex.

In a preferred implementation form of this invention, the proportion of zinc in the sugar alcohol mixture is 0.5-10 mg zinc per g of sugar alcohol, preferably 1-5 mg zinc per g of sugar alcohol. This invention also concerns the use of the afore-mentioned sugar alcohol mixtures, particularly of 1,1-GPM and 1,6-GPS, preferably in combination with zinc and/or 1,1-GPS, to prepare a drug which acts in an immuno-stimulating and/or antimicrobial manner.

This invention accordingly concerns also products, in particular drugs, foods and stimulants, which contain the afore-mentioned sugar alcohol mixtures, especially sugar alcohol mixtures containing 1,1-GPM and 1,6-GPS in conjunction with zinc, in particular organic and inorganic zinc salts. In an especially preferred implementation form of the invention, the weight ratio of the sugar alcohols available in the product is almost 1:1, i.e. the sugar alcohol mixture of 1,1-GPM and 1,6-GPS contained in the product is isomalt. However, in accordance with the invention, provision also can be made that the sugar alcohol mixture consists of 1,1-GPM, 1,6-GPS and 1,1-GPS, or that it contains a mix of these sugar alcohol mixtures, if necessary together with residual amounts of other sugar alcohols and oligosaccharides.

The products according to the invention can exist in the form of oral application styles such as pastilles, tablets, compressed lozenges, syrups, dragees, hard or soft caramels or as suspensions, sprays, inhalation mixtures or similar.
The use according to the invention, or the products according to the invention, are distinguished therefore by a particularly dental-friendly matrix in the form of the sugar alcohol mixture of 1,1-GPM and 1,6-GPS to be used according to the invention, if necessary in combination with 1,1-GPS, in which the zinc$^{2+}$ ions possibly available are not hindered in their effectiveness against cold illness-causing microorganism such as rhinoviruses, or as an immuno-stimulant, by forming complexes. The products according to the invention, in particular implemented as a therapeutic anti-cold preparation, or the use according to the invention is distinguished moreover by its low dissolution rate in comparison with known products, so that the active ingredient is released and can act over a long period at the desired place of action. Finally, the sugar alcohol mixtures to be used according to the invention are calorie-reduced, even suitable for diabetics, and allow the preparation of products with good storage properties, so that a less costly packaging material is possible. Because of their improved shelf life, for example compared to products containing sugar, the products according to the invention are particularly suitable also for use in humid tropical regions, which are often regions with a zinc deficiency and high risk of infection.

In a further form of implementation, the invention also concerns the afore-mentioned uses and the afore-mentioned products containing zinc, the sugar alcohol mixture being either a 1,6-GPS-enriched or a 1,1-GPM-enriched mixture, as described in DE 195 32 396 C2. Such a 1,6-GPS-enriched mixture can contain 1,6-GPS and 1,1-GPM in a ratio from 57% by weight : 43% by weight up to 99% by weight : 1% by weight (based on DS). Such a 1,1-GPM-enriched mixture can for example contain 1,6-GPS and 1,1-GPM in a ratio from 1% by weight : 99% by weight up to 43% by weight : 57% by weight (based on DS). Through the variation of the ratios to each other of the sugar alcohol quantities used, and made possible according to the invention, it is possible to adjust the desired dissolution rate of the products prepared. Depending on the desired application and active ingredient to be administered, a particular ratio of the sugar alcohol mixtures can be adjusted, so that virtually any desired dissolution rate and therefore release rate of the pharmacologically-active component, especially zinc, can be set. This is based on the dissolution kinetics of the sugar alcohols 1,6-GPS, 1,1-GPM and 1,1-GPS differing considerably from one another. For instance, 1,1-GPM distinguishes itself by a markedly lower dissolution rate than 1,6-GPS or other substances being considered for this application. In cases in which a rapid release of the active ingredient, in particular zinc, is desired, 1,6-GPS-enriched mixtures according to the invention with their elevated dissolution rate are used, whereas in cases in which a chronologically extended release of the pharmaceutical agents is important, 1,1-GPM-enriched mixtures are primarily used.

Accordingly the invention in a preferred form of implementation concerns the use of a sugar alcohol mixture of at least 98% by weight 1,6-GPS and 1,1-GPM, in which are contained 75 to 85
% by weight 1,6-GPS and 25 % by weight 1,1-GPM and which is described as isomalt GS. In addition, small amounts of mannitol and sorbitol, for example in each case 0.5 % by weight, can be present in the sugar alcohol mixture.

In another preferred form of implementation, a sugar alcohol mixture can be provided which contains more than 90 % by weight 1,1-GPM and 1,6-GPS relative to the total quantity of the mixture, in which maximum quantities of mannitol (3 % by weight) and sorbitol (6 % by weight) can be provided. An isomalt mixture of this type is described as isomalt HC.

In another preferred form of implementation, a sugar alcohol mixture is provided which has more than 95 % by weight 1,6-GPS and 1,1-GPM, 43 to 57 % by weight of it being 1,6-GPS. Provision is made in this form of implementation for maximum amounts of mannitol (1 % by weight) and sorbitol (2 % by weight) to be provided. Such an isomalt is described as isomalt HB.

Finally in another preferred form of implementation, a sugar alcohol mixture is provided which has more than 98 % by weight 1,6-GPS and 1,1-GPM, 75 to 85 % by weight of it being 1,1-GPM and 25 to 15 % by weight being 1,6-GPS. In such a form of implementation, maximum amounts of mannitol (0.5 % by weight) and sorbitol (0.5 % by weight) are provided. This sugar alcohol mixture is described as isomalt GS.

The afore-mentioned sugar alcohol mixtures can be used in the application provided according to the invention and in the drugs provided according to the invention in an advantageous way, particularly preferred being the use of no additional sweetening agent, sugar or sugar substitute.

In a particularly preferred form of implementation of the invention, provision is made that the products according to the invention, or that is the sugar alcohol mixture of 1,1-GPM and 1,6-GPS used according to the invention, contain in addition intensive sweetening agents such as ascesulfame K, aspartame, cyclamate, glycyrrhizin, neotame, neohesperidine DHC, stevioside, sucralose, thaumatin, saccharin or similar. In an advantageous way, the products according to the invention, or that is the sugar alcohol mixture of 1,1-GPM and 1,6-GPS used according to the invention, contain in addition tastes or flavourings such as lemon or peppermint flavourings. The products according to the invention, or the sugar alcohol mixture of 1,1-GPM and 1,6-GPS used according to the invention, can also contain food-compatible acids such as ascorbic acid, malic acid or gluconic acid as well as lubricant fatty acids or their salts like magnesium stearate or sodium stearate.

Finally, provision can be made that artificial colourings and/or water-soluble agents such as modified starches, polyvinyl pyrrolidone (PVP) or carbomethylcellulose are contained in the products according to the invention, or the sugar alcohol mixture of 1,1-GPM and 1,6-GPS used according to the invention. A mixture of this type is described as isomalt GS.
According to the invention, apart from the sugar alcohol mixture of 1,1-GPM and 1,6-GPS provided according to the invention, the products or applications in a preferred form of implementation in addition can also include enzymes, coenzymes, minerals, vitamins, antibiotics, nicotine, caffeine, eucalyptus, codeine, phenacetin, paracetamol, acetylaminoephensols, acetylsalicylic acid, menthol or other pharmacologically-active agents (cf. Smit M.B.H. and Feldmann W. "Over-the-counter cold medications; see: A critical review of clinical trials between 1950 and 1991", in: JAMA 269, (1993) 2258-2263). The pharmacologically-active agents, in particular zinc, can be provided in an amount so that they cause the desired pharmacological effect.

The invention in a preferred form of implementation also provides that the foods, drugs or stimulants containing 1,1-GPM and 1,6-GPS (especially also zinc, as well as 1,1-GPS if required and possibly other afore-mentioned substances) are completely or essentially sugar-free, that is in particular free of saccharose and/or fructose and/or glucose. "Essentially sugar-free" is understood to mean that a maximum of 10% by weight, preferably a maximum of 5% by weight, and especially preferred a maximum of 1% by weight of sugar (relative to the total weight of the product) are available.

In the preferred form of implementation, the products according to the invention distinguish themselves by their freedom from sugar, they are dentally-protective, non-cariogenic and in addition do not promote the formation of plaque matrices. In addition the products according to the invention are suitable for diabetics, in contrast to easily digestible products containing glucose, since isomalt is only slowly and incompletely decomposed by small intestine enzymes and therefore the blood glucose level hardly increases or increases not at all. Glycaemic index and insulin response are correspondingly low. In addition, isomalt reaching the colon is fermented to short-chain fatty acids and therefore counteracts constipation, from which in particular older people frequently suffer.

Further advantageous form of the invention are given by the sub-claims.

The invention is explained in more detail with reference to examples and associated figures, which show:

in Figure 1 the effect of isomalt and zinc salt as well as standard substances on the growth of Staphylococcus aureus and

in Figure 2 the mortality behaviour of Staphylococcus aureus in 60% isomalt solution (80 parts of 1,6-GPS : 20 parts of 1,1-GPM).
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Examples

Example 1: Antibacterial effect of isomalt

Staphylococcus aureus was cultivated at 37°C in CASO medium to which, apart from the
carbon source glucose (5 %), additional isomalt (0.58 M) had been added. The growth of the
bacterial culture in the agitating retort was determined using the refractive index (RI
1574) after 16 h
and compared to a control (CASO medium without isomalt). In all experiments it was observed that
the growth of the cultures set in isomalt was inhibited by more than 50 %. Compared to that, the
growth in equimolar quantities of sorbitol (0.58 M) was merely reduced to approximately 75 % (see
Figure 1, specimens 1, 2 and 5). Furthermore, an antibacterial action of a 60 % 1,6-GPS-enriched
solution (80/20 parts of 1,6-GPS/1,1-GPM) could be detected (see Figure 2 for the mortality
behaviour of Staphylococcus aureus).

The highly concentrated 60 % isomalt solution was contaminated with about 1000 germs of
various bacteria (Staphylococcus aureus and Streptococcus pneumoniae) and specimens were
taken at different times. The germs surviving in the solution were made visible by filtration through a
filter (0.45 µm) and incubation of the filter on selective agar culture mediums. It could be shown
that after 30 min, the germ count was considerably reduced and after one day (1140 min) bacteria
were almost no longer detectable.

Example 2: Antibacterial effect of zinc/isomalt

Isomalt hard caramels, in which zinc gluconate or zinc acetate (2.5 mg zinc per g isomalt)
had been incorporated as well, were dissolved 20 % (w/v) in CASO medium and inoculated with a
fresh Staphylococcus aureus culture. The growth of the bacteria at 37°C in the agitating retort was
determined using the refractive index (RI
1574) after 16 h and compared to a control (CASO medium
or hard caramels). Neither the combination isomalt/zinc gluconate nor isomalt/zinc acetate allows
growth of the bacteria. In all cultures, refractive indices of 0.0 were measured (see Figure 1,
specimens 3 and 4).

Example 3: Determination of free Zn ions in sugar solutions, sugar alcohol solutions and hard caramel
solutions

10 ml of zinc gluconate solution (2 g zinc gluconate in 250 ml demineralised water) are mixed
with 10 ml of polyol solution (1.0 g sugar or sugar alcohol dissolved in 25 ml demineralised water)
and 2 ml of acetate buffer (pH = 6.0) and warmed to 35°C in a bulb.

Then a 1 molar NaHCO₃ solution is added in small quantities in drops and the turbidity
determined by means of a turbidity photometer.

Isomalt, saccharose, glucose, isomalt hard caramels, the zinc gluconate used as the zinc
source, as well as two commercial products are compared in the table, the total quantity of zinc
ions corresponding in each case to 28 mg in 50 ml.
Free zinc ions and their percentage (of the total quantity) indicate that in the presence of isomalt (also as hard caramel) comparatively there are many free zinc ions compared to other polyols.

Table: Nephelometric determination of free Zn ions in the presence of polyols

<table>
<thead>
<tr>
<th>Sugar/sugar alcohol/hard caramel</th>
<th>Zn ions free in solution* (in mg in 50 ml solution)</th>
<th>Percentage of free (non-complex) ions (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isomalt</td>
<td>15.11</td>
<td>58</td>
</tr>
<tr>
<td>Glucose</td>
<td>13.00</td>
<td>50</td>
</tr>
<tr>
<td>Saccharose</td>
<td>12.40</td>
<td>48</td>
</tr>
<tr>
<td>Isomalt/hard caramels</td>
<td>10.40</td>
<td>40</td>
</tr>
<tr>
<td>Hard caramels commercial product A</td>
<td>3.90</td>
<td>15</td>
</tr>
<tr>
<td>Hard caramels commercial product B</td>
<td>3.80</td>
<td>15</td>
</tr>
<tr>
<td>Zn gluconate</td>
<td>14.62</td>
<td>64</td>
</tr>
</tbody>
</table>

*Normalised to equal concentrations of Zn ions

(Commercial product A: hard caramel of saccharose syrup and glucose syrup mixed with zinc gluconate;
Commercial product B: hard caramel of saccharose syrup and glucose syrup mixed with zinc gluconate and lemon flavour.)

Example 4: Antirhinoviral activity of Isomalt zinc preparations
Starting materials and testing method.

Four different zinc concentrations (0.125 mM, 0.1 mM, 0.075 mM and 0.05 mM), in the form of zinc gluconate and zinc acetate together with 5 mg/ml isomalt (equimolar mixture of 1,1-GPM and 1,6-GPS), were tested with regard to their possible antiviral activity. Three identical preparations for 3 different rhinovirus serotypes, as well as in each case a control with the inclusion of EDTA (in an equimolar amount of the zinc salt), were made for each of the combinations. Additional controls contained isomalt without zinc salt and each zinc salt concentration without isomalt.

The preparations mentioned were tested for their effect on rhinoviruses in HeLa cells (obtained from ATCC Rockville MD, Catalogue No. CRL-1958). The HeLa line was cultivated in Dubelco's modified Eagle medium (DMEM), supplemented with foetal cattle serum (5 % final concentration) and gentamicin sulphate (50 μg/ml final concentration). The cell cultures were incubated at 37°C in a moist 5 % CO₂ atmosphere. On the presence of the virus, the incubation temperature was reduced to 33°C.
The rhinovirus strains were allowed to grow on the HeLa cells and the TCID_{50} (titre with 50 % tissue culture infectivity) determined by serial dilution.

The cells were harvested by treating with trypsin, counted and dispersed in a concentration of 25,000 cells per well in each well of a 96-well micro-titration plate. After inoculation, the plates were incubated overnight at 37\(^\circ\) C in a moist 5 % CO\(_2\) atmosphere and next morning the medium removed from the cells and replaced with 175 \(\mu\)l of a 1.143 x concentration of the corresponding test substance combination. The plates were incubated at 37\(^\circ\) C in a moist 5 % CO\(_2\) atmosphere for one hour.

After this incubation, for the evaluation of the antiviral activity, the wells were treated in each case with 25 \(\mu\)l of a DMEM solution with 100 TCID_{50} of the respective virus. On each plate, 6 wells were used as a virus control (without zinc isomalt preparation) and another 6 wells used as a cell control (without zinc isomalt preparation and without a virus). The plates were then incubated at 33\(^\circ\) C in a moist 5 % CO\(_2\) atmosphere and examined under a microscope for cytopathogenic effects.

Results:

The antiviral activity was defined as the reduction in the cytopathogenic effect of rhinoviruses in HeLa cells. The determination was performed microscopically, or that is using colorimetric tests. Determination was carried out at the time at which the virus control (without addition of zinc isomalt preparations) indicated the complete symptomatic of a viral cytopathogen effect. For the rhinovirus Type 7 the evaluation of antirhinoviral activity was carried out three days after infection, and four days after infection for serum Types 1A and 2. The cell controls (HeLa cells without virus) showed no symptoms of a virus infection, whereas the virus controls (HeLa cells with virus and without zinc isomalt preparation) showed the complete symptomatic of a virus infection.

The viral infection of HeLa tissue cultures led to histological changes in the cells. These could be observed microscopically for example by a change in the cell shape and desorption from surfaces. In the course of a viral infection the cells can be destroyed and/or removed from the substrate. The manifestation of the symptomatic of a viral infection was analysed both microscopically and by staining techniques. During the stain analysis, advantage was taken of removing and rinsing infected cells from the substrate. Wells with cells, which are protected against viral infection, here have a cell coating coloured in stain tests darker than wells with unprotected cells, since in the latter there are fewer cells which could contribute to staining of the cell coating.

Against the rhinovirus Type 1a, antiviral action against infection by rhinovirus Type 1A was observed with 0.1 mM zinc gluconate in conjunction with isomalt. Just as antiviral activity against the rhinovirus Type 1A was obtained for 0.1 mM of zinc acetate in conjunction with isomalt and 0.075 mM zinc gluconate in conjunction with isomalt. These activities were, however, less than that obtained with 0.1 mM of zinc gluconate in conjunction with isomalt.
Against the rhinovirus Type 2, an antiviral effect was obtained with 0.1 mM of zinc acetate together with isomalt, just as with 0.1 mM of zinc gluconate in conjunction with isomalt, and, in each case to a lesser degree, with 0.075 mM of zinc gluconate in conjunction with isomalt and 0.1 mM of zinc gluconate without isomalt.

Against the rhinovirus Type 7, in each case with 0.05, 0.075 and 0.1 mM zinc acetate in conjunction with isomalt, and 0.05 mM of zinc gluconate in conjunction with isomalt, an antiviral effect was observed which corresponded in degree approximately to the effect of 0.075 mM of zinc gluconate in conjunction with isomalt against rhinovirus 2.

The antiviral activities against rhinovirus Type 1a and Type 2 could be raised by addition of EDTA in equimolar quantities of the zinc salt. This verifies the "free ions theory" discussed in the "Description" introduction. Zinc acetate and zinc gluconate on their own produce only a comparatively weak, characteristic antiviral protection with 0.1 mM of zinc gluconate (without isomalt). However, the protection obtained with 0.1 mM of zinc gluconate was significantly weaker than the protection achieved with 0.1 mM of zinc acetate or zinc gluconate, each in conjunction with isomalt.

The data show that pharmaceutical preparations containing isomalt and zinc gluconate or zinc acetate offer better protection against rhinoviral infections than zinc preparations on their own.
Claims

1. Use of a sugar alcohol mixture containing 1,6-GPS-(6-O-D-glucopyranosyl-D-sorbitol) and 1,1-GPM-(1-O-D-glucopyranosyl-D-mannitol) in foods, drugs and stimulants as a therapeutic active ingredient.

2. Use according to claim 1, in which the therapeutic active ingredient is an antimicrobial agent.

3. Use according to claim 1, in which the therapeutic active ingredient is an immunostimulant.

4. Use according to any one of the preceding claims, in which 1,6-GPS and 1,1-GPM are present in the sugar alcohol mixture in almost equimolar quantities.

5. Use according to any one of claims 1 to 3, in which the sugar alcohol mixture is a 1,6-GPS-enriched mixture or a 1,1-GPM-enriched mixture.

6. Use according to claim 5, whereby 75 to 85% by weight of 1,6-GPS and 25 to 15% by weight of 1,1-GPM are contained in the sugar alcohol mixture.

7. Use according to claim 5, whereby 75 to 85% by weight of 1,1-GPM and 25 to 15% by weight are contained in the sugar alcohol mixture.

8. Use according to any one of claims 1 to 3 or claims 5 to 7, in which the sugar alcohol mixture additionally contains 1,1-GPS (1-O-D-glucopyranosyl-D-sorbitol).

9. Use according to any one of the preceding claims, in which the sugar alcohol mixture additionally contains zinc.

10. Use according to any one of the preceding claims, in which the zinc is present in the form of organic or inorganic zinc salts.

11. Use according to any one of the preceding claims, in which the zinc salt is zinc gluconate or zinc acetate.

12. Use according to any one of the preceding claims, in which the sugar alcohol mixture additionally contains artificial colourings, flavours, tastes, edible acids or small proportions of other sugar alcohols.

13. Use according to any one of the preceding claims, in which the food, stimulant or drug is a hard caramel, soft caramel, compressed lozenge, dragee, pastille or chewing gum.

14. A drug for human or animal consumption, containing 1,1-GPM and 1,6-GPS as well as 0.5 - 10 mg zinc per one gram of sugar alcohol.

15. A drug according to claim 14, the drug additionally containing 1,1-GPS.

16. A drug according to either one of claims 14 and 15, the drug containing 1,1-GPM and 1,6-GPS in almost equimolar quantities.

AMENDED SHEET
17. A drug according to either one of claims 14 and 15, the drug containing 1,1-GPM and 1,6-GPS in the form of a 1,1-GPM-enriched mixture or a 1,6-GPS-enriched mixture.

18. A drug according to claim 17, the drug containing 75 to 85 % by weight of 1,1-GPM and 25 to 15 % by weight of 1,6-GPS.

19. A drug according to either of claims 14 and 15, the drug containing 15 to 25 by weight % of 1,1-GPM and 75 to 85 % by weight of 1,6-GPS.

20. A drug according to any one of claims 14 to 19, in which zinc is present in the form of inorganic or organic zinc salts.
ZnGlu = Zinc gluconate
ZnAc = zinc acetate

Fig. 1
Mortality behaviour of Staphylococcus aureus

Fig. 2

REPLACEMENT SHEET (REGULATION 26)