Antiviral Action of a Homeopathic Medication

Horst Metelmann
Dr. rer. nat. Bernadette Glatthaar-Saalmüller
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Abstract

Euphorbium compositum® S, a homeopathic combination preparation available in the form of drops, nasal spray, and injection solution, is prescribed for inflammation of the mucosae of the nose and sinuses. Infections in these areas are primarily viral in origin, although bacterial superinfections are also common. An *in vitro* study examined the effect of Euphorbium compositum® S against pathogens causing various viral infections-influenza A, respiratory syncytial virus (RSV), and herpes simplex virus type 1 (HSV-1). HSV infections play a major role in so-called superinfections in common colds. This study shows that *in vitro*, Euphorbium compositum® S has antiviral effects against RSV and HSV-1. In addition, an antiviral effect against influenza A virus, although minimal, was also noted.

Resumen

La preparación homeopática Euphorbium compositum® S, disponible en gotas, nebulizador, y solución inyectable, se prescribe para inflamaciones de las mucosas del nariz y de los senos paranasales. Las infecciones en aquellos sitios son fundamentalmente del origen viral, aunque existan también sobreinfecciones bactéricos. Un estudio *in vitro* examinó el efecto de Euphorbium compositum® S contra los agentes patógenos que causan una variedad de infecciones virales - influenza A, virus respiratorio sincitial (RSV), y el virus de herpes simplex tipo 1 (HSV-1). Las infecciones HSV tienen mucha importancia en las sobreinfeccio... en los resfriados comunes. ... ...dio demostró que *in vitro*, Euphorbium compositum® S tiene efectos antivirales contra los virus RS y HSV-1. También se pudo notar un efecto antiviral, aunque mínima, contra el virus influenza A.

Introduction

From the medical perspective, viral illnesses have increased in importance in recent years.[1] In fighting bacterial infections, there is a broad palette of antibiotics to choose from, but only a few effective antiviral drugs are available. Medications for treating upper and lower respiratory tract infections are urgently needed. Most of the few antiviral medications are nucleoside analog inhibitors (antimetabolites) that specifically inhibit replication of viral nucleic acids. These drugs are highly effective against viruses but may also cause severe adverse effects.

At present, the most successful and effective antiviral medication is acyclovir (Zovirax®), which inhibits the replication of herpes viruses.[2-4] It is especially effective when applied topically to herpes fever blisters. Although the effects of acyclovir are for the most part restricted to virus-infected cells, it is highly toxic.[5,6] Furthermore, it does not affect latent herpes viruses that persist in the ganglia, so relapses occur whenever treatment is discontinued. It is expected that herpes viruses will become resistant to acyclovir with long-term treatment.

Ribavirin (Virazole®)—also a nucleoside analog inhibitor—has been known since 1970 for its antiviral effect against respiratory syncytial virus (RSV) infections.[7] However, it too is highly toxic, and it can cause anemia when administered orally.[10,11] Since 1986 ribavirin has been commercially available in aerosol form for use in RSV infections: the inhaled drug is appl...

directly to the affected tissues without first passing through the bloodstream.[12,13]

Amantadine (amantadine hydrochloride), a cyclic amine, was first made available in 1966 under the trade name Symmetrel® for prophylactic use and oral treatment of viral influenza in the first 24 hours of illness.[14-20] The drug was poorly tolerated, however, and has been withdrawn from the market.

The present study investigates the antiviral action of Euphorbium compositum® S under *in vitro* conditions, specifically, its effect on influenza A and RSV, two of the pathogens causing respiratory tract infections. Influenza A, the virus causing "classic flu," belongs to the family of orthomyxoviruses, while RSV, which is responsible for infections of the upper and lower respiratory tract, is a paramyxovirus. Both are single-strand, coated RNA viruses. An additional test investigated the effect of the medication on herpes simplex virus type 1 (HSV-1), the coated DNA virus that causes fever blisters.

- Euphorbium
- Pulsatilla pratensis
- Luffa operculata
- Hydrargyrum bijodatum
- Mucosa nasalis suis
- Hepar sulfuris
- Argentum nitricum
- Sinusitis Nosode

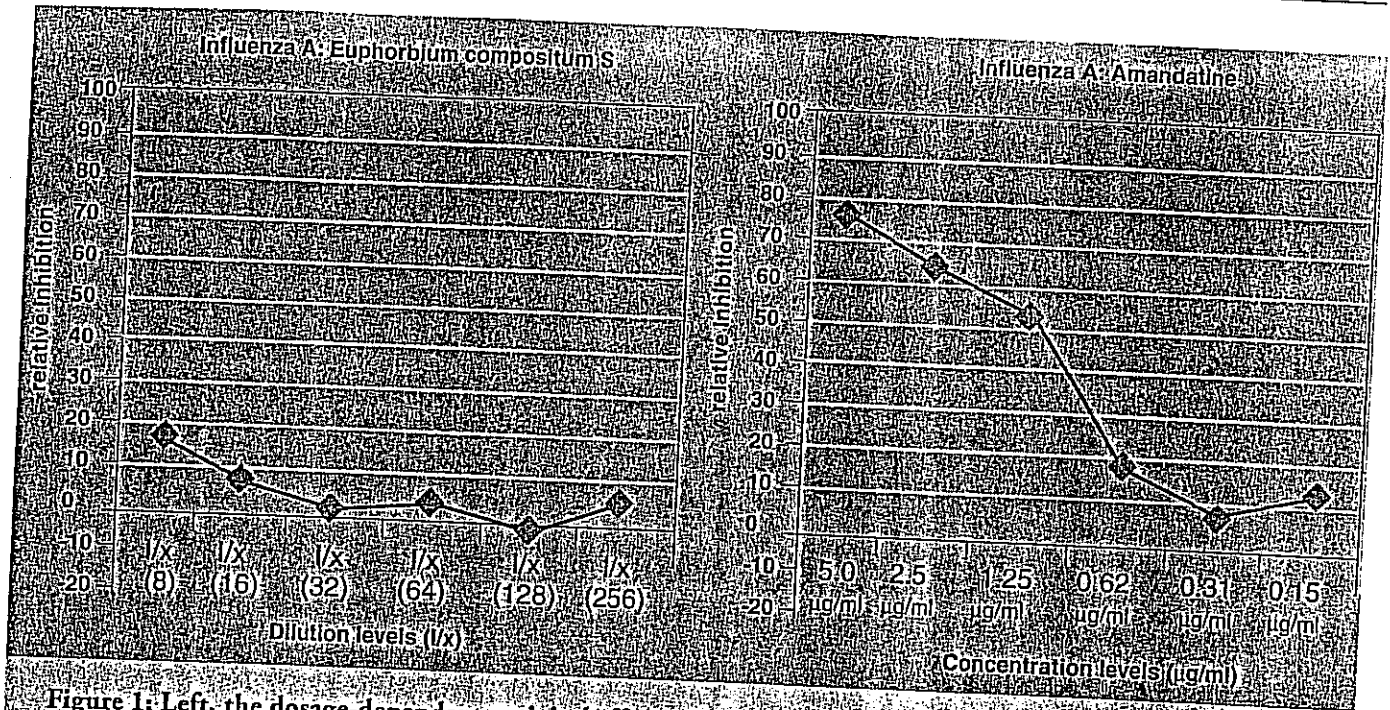Table: Composition of Euphorbium compositum® S

Figure 1: Left, the dosage-dependent antiviral effect of the total preparation Euphorbium compositum® S on Madine-Darbine canine kidney cell cultures infected with influenza A virus, compared with the effect of the reference substance amantadine (1-amantadine hydrochloride, right). The relative standard deviations (%) for all values = <10%.

Materials and Methods
The Test Substances

Euphorbium compositum® S consists of 8 ingredients (Table). It was supplied by the manufacturer, Biologische Heilmittel Heel GmbH, Baden-Baden, Germany. To verify that our *in vitro* systems were suitable for testing the antiviral action of Euphorbium compositum® S, the medications described here (acyclovir, ribavirin, and amantadine) were used as reference substances (positive controls).

Cells and Viruses

The influenza A/Chile-1/83(H1N1) virus was isolated from nasal mucosal secretions and identified by means of immunofluorescence.[21] Madine-Darbine canine kidney (MDCK) cells were used to culture this virus. The cells were grown in serum-free Minimal Essential Earle's Medium (MEM/Earle's) with the addition of 1-µg/mL trypsin, 2-mmol/L glutamine, 100-U/mL penicillin, and 0.1-mg/mL streptomycin.

Long-strain RSV was isolated from nasal and throat secretions and Thea strain HSV-1 was isolated from fluid used for gargling. Both strains of viruses were supplied by the Department of Medical Virology and Epidemiology of the Hygiene Institute of the University of Tübingen. The viruses were identified by means of a specific antibody (apiBiomerieux, Nürtingen, Germany). Human epithelial (human epidermoid carcinoma, or HEp-2) cells were grown in Dulbecco's Modified Medium with the addition of 2% fetal calf serum, 2-mmol/L glutamine, 100-U/mL penicillin, and 0.1-mg/mL streptomycin.

The cell strains were incubated together with the respective virus dilution series in cell culture plates with either 6 or 12 depressions for one hour at 34°C. After removing the virus inoculum, the cell cultures were coated with a virus-specific medium containing either 0.6% indubiose or 1% carboxylmethyl cellulose. Virus-caused plaques could be counted 5 to 8 days later. The concentration of plaques was calculated according to methods previously described[22] and recorded in plaque-forming units.

Determining the Cytotoxicity of the Test Substances

To establish the highest noncytotoxic concentration of the test substances, MDCK and HEp-2 cells were cultured alone for three days and then for five additional days in various concentrations of Euphorbium compositum® S or one of the following reference substances: amantadine (amantadine hydrochloride; ratiopharm, Ulm/Donautal) for influenza A; ribavirin (Virazole®; ICN Pharmaceuticals, Frankfurt), for RSV; and acyclovir (Zovirax®, Deutsche Wellcome GmbH, Burgwedel), for HSV-1. Cells cultivated in an unamended medium served as the control in the cytotoxicity tests.

Microscopic evaluations of changes in cell morphology, marking with fluorescein diacetate and ethidium bromide, and an enzymatic test modified according to the methods of Noll et al[23-25] were used to analyze cytotoxicity.
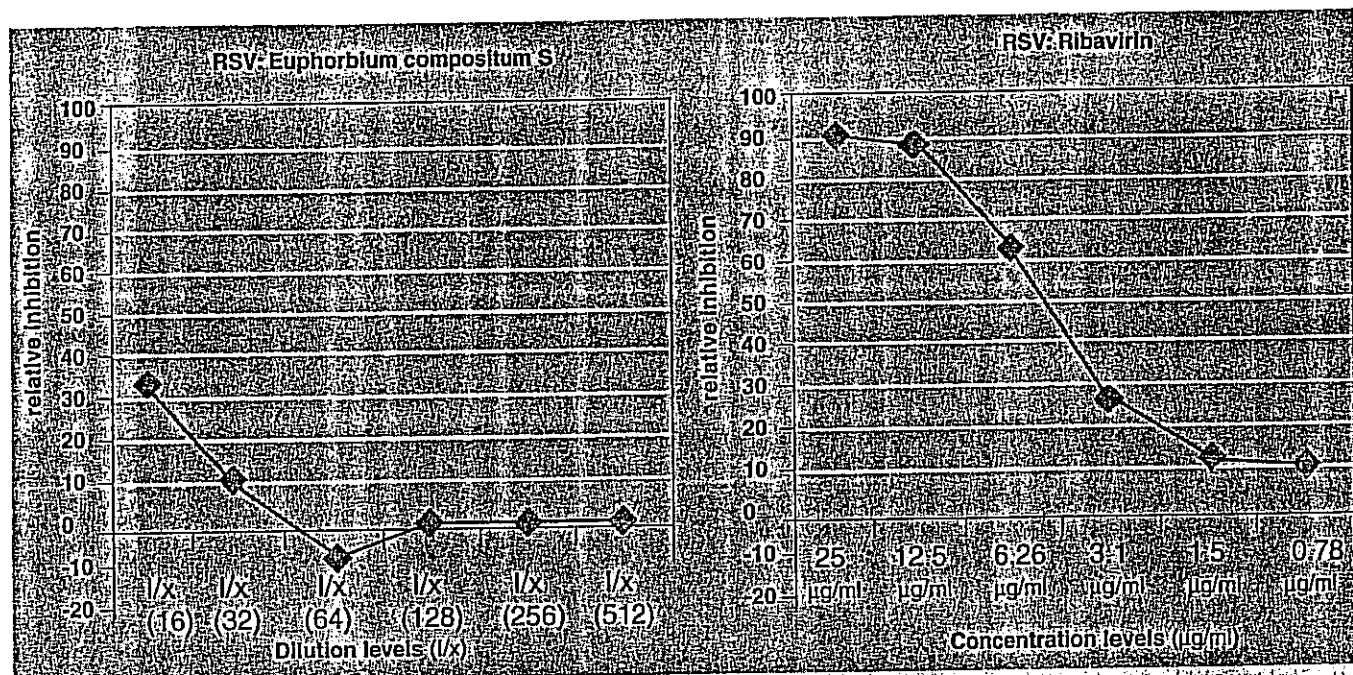
Figure 2: Left, the dosage-dependent effect of the total preparation Euphorbium compositum® S on human epidermoid carcinoma 2 cell cultures infected with respiratory syncytial virus (RSV), compared with the effect of the reference substance ribavirin (Virazole®, right). The relative standard deviations for all values = <10%.

Antiviral Activity

Plaque reduction assays were used to determine the antiviral activity of the test substance.[26-28] The single-layer cell culture method we used allows infectious particles injected onto the cell layer to multiply at the infection site, spreading to neighboring cells after lysis of the initially affected host cells. Eventually, clearly defined, local cytopathogenic changes in the cell layer—so-called lytic plaques—develop. The free spread of the infection and therefore also the development of secondary plaques is prevented by coating the infected cell layer with a viscous colloidal medium. Adding the test substance to the coating medium permitted us to assess the direct influence of the test substance on plaque development due to the virus.

For this purpose, confluent monolayers of MDCK and HEp-2 cells in cell-culture plates with 6 depressions (MEM Earle's with the addition of 10% FKS) were infected with the virus strains with a multiplicity of infection of 0.1. The concentration of the respective viruses had been determined in prior experiments. Infection was allowed to occur for one hour at 34°C (MEM Earle's without FKS). The cell layers were then washed and coated with a viscous colloidal medium (0.6% indubiose or 1% carboxymethyl cellulose) containing noncytotoxic concentrations (as determined by the cytotoxicity tests) of Euphorbium compositum® S or of the reference substances. Culturing then continued until lesions (plaques) became visible in the cell layer of the untreated control (MEM/virus control). For the influenza A virus, this process took 5 days; for RSV, 8 days; for HSV-1, 6 to 7 days. The cell layers were then fixed with 5% formalin and stained with Giemsa solution. The plaques, which appeared as transparent holes in the cell layers, could then be counted when the cultures were held over a light source.

The antiviral effects were quantified by counting the plaques in six replications each from two separate test batches for each test substance. The number of plaques in the untreated virus control was defined as a 100% positive infection, so that the inhibiting effects of the test substances could be expressed as percent inhibition.

Results
Cytotoxicity Tests

Our objective was to ascertain the *in vitro* cytotoxicity of Euphorbium compositum® S to select the highest possible concentration that could be used in antiviral tests to determine dosage-dependent effects. Microscopic evaluations of changes in cell morphology, fluorescein diacetate marking, uptake of ethidium bromide, and results of an enzymatic test were used to analyze cytotoxicity.

The cytotoxicity tests showed that Euphorbium compositum® S does not compromise the vitality of MDCK cells when diluted at least 1:8, while 1:16 dilutions do not harm HEp-2 cells. These noncytotoxic concentrations were then used to determine the antiviral activity of the test substance.
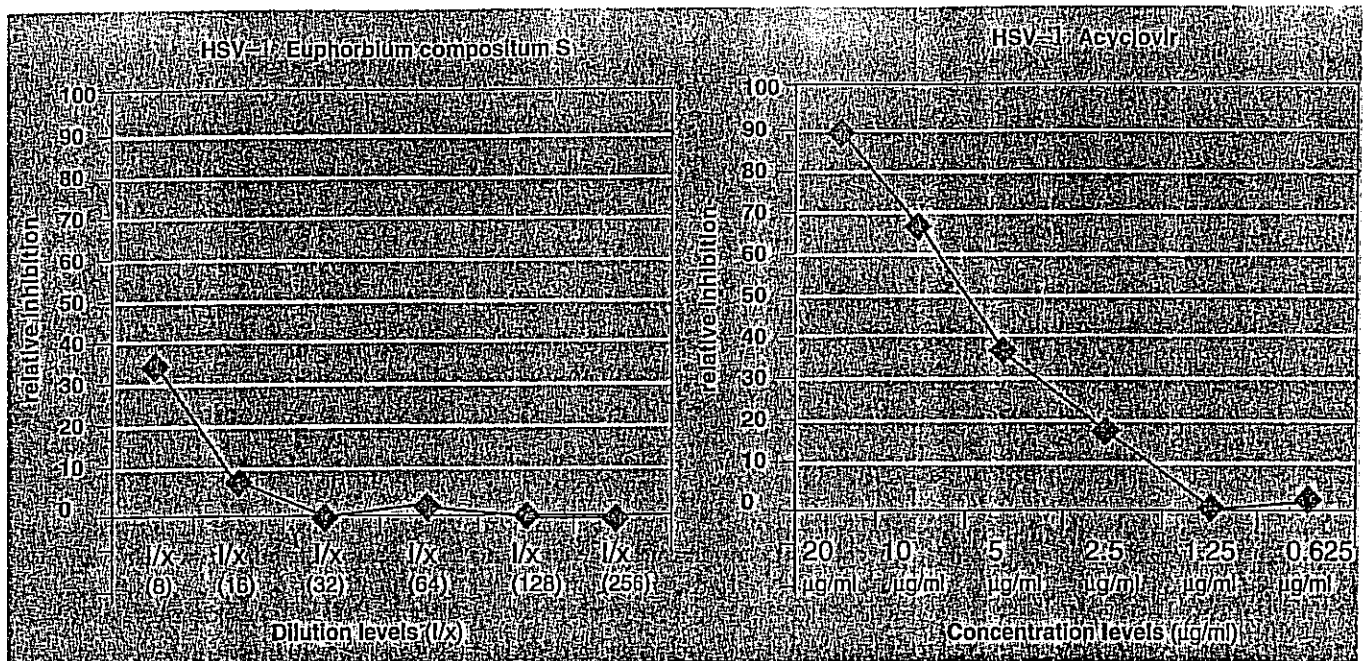
Figure 3: Left, dosage-dependent effect of the total preparation Euphorbium compositum® S on human epidermoid carcinoma 2 cell cultures infected with herpes simplex virus type 1 (HSV-1), compared with the effect of the reference substance acyclovir (Zovirax®, right). The relative standard deviations for all values = <10%.

Determining Antiviral Activity

Our goal was to investigate possible antiviral effects of Euphorbium compositum® S. Testing was accomplished by infecting MDCK cells with the influenza A virus and HEp-2 cells with RSV and HSV-1 (Figures 1-3). To verify the suitability of the *in vitro* test systems, reference substances with known antiviral effects were included in the tests.

A plaque reduction assay that measured the ability of the test substance to reduce virus-caused plaque formation was used to prove antiviral activity. The dosage-dependent activity was quantified by counting the virus-caused plaques. Substance-specific activity was calculated in comparison to the controls (noninfected cells and infected cells cultured in an unamended medium).

In comparison to the controls, Euphorbium compositum® S showed no significant activity against influenza A viruses, although a minimal effect could be surmised at the highest possible concentration (a 1:8 dilution) (Figure 1). The efficacy of amantadine against influenza A viruses was confirmed by this *in vitro* test system. Euphorbium compositum® S showed considerable activity against RSV at its strongest noncytotoxic dilution level (1:16), causing an almost 35% reduction in the number of virus plaques (Figure 2). At the next level of dilution, the effect was weaker but still present. The antiviral activity of the reference substance ribavirin was confirmed by comparison tests.

It is interesting to note that the test substance had an almost equally strong effect on HSV-1 (Figure 3). At its highest noncytotoxic concentration (a 1:8 dilution), the antiviral effect of Euphorbium compositum® S was readily apparent and reduced HSV-1 plaques by approximately 30%. Once again, tests confirmed the efficacy of the reference substance acyclovir.

Discussion

Our findings clarify the antiviral effects of the homeopathic preparation Euphorbium compositum® S. Established virological *in vitro* test systems were used to prove that the product has significant effects against RSV and HSV-1. Although its antiviral activity is clearly weaker than that of the reference substances used to confirm the reliability of the *in vitro* model, the homeopathic medication also has far fewer adverse effects on the total organism than the synthetic substances.

Since the reference substances amantadine, ribavirin, and acyclovir were undiluted substances known for their strong antiviral effects, it is remarkable that the highest concentrations of Euphorbium compositum® S inhibited the infectivity of RSV and HSV-1 strains by approximately 30% in comparison with the untreated controls. This raises the question of whether higher concentrations of a potential active ingredient in the test preparation (a combination remedy) might have still stronger antiviral effects on RSV, HSV-1, and influenza A viruses. We were not able to test this hypothesis because the components of Euphorbium compositum® S were not available separately. Further study is recommended to ascertain the effects of

individual ingredients and any possible additive effects of Euphorbium compositum® S when combined with other substances.

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For the authors:

Dr. rer. nat. Bernadette Glatthaar-Saalmüller
Glatthaar Laboratory for Virological Testing, Tissue Culturing, and Immunological Diagnostics
Scientific and Medical Institute of the University of Tübingen/Reutlingen
Markwiesenstraße 55
D-72770 Reutlingen
Germany