

In vitro evaluation of the antiviral effects of the homeopathic preparation Gripp-Heel on selected respiratory viruses¹

Bernadette Glatthaar-Saalmüller

Abstract: Gripp-Heel[®] is a homeopathic preparation frequently used in the treatment of respiratory viral infections such as various types of influenza and the common cold. The antiviral activity of Gripp-Heel was studied in vitro on human pathogenic enveloped and nonenveloped RNA and DNA viruses. Before the antiviral assays, in vitro cytotoxicity of Gripp-Heel was determined with cells used for the infection experiments (HeLa, HEP-2, MDCK, BGM) as well as with mitogen-stimulated peripheral blood mononuclear leukocytes. A concentration of 0.5 of the commercially available product slightly reduced cell viability and proliferative capacity, and experiments on antiviral activity were determined starting with a dilution of 0.2 of the commercially available product. The antiviral activity was determined against a broad panel of enveloped and nonenveloped DNA and RNA viruses with plaque reduction assay, cytopathogenic assays, virus titrations, analysis of the viral proteins in virus-specific enzyme immunoassays, and haemagglutination tests. Control substances were acyclovir (10 µg/mL), ribavirin (6 µg/mL), and amantadine hydrochloride (5 µg/mL), depending on the virus type. Gripp-Heel demonstrated dose-dependent in vitro activity (significant reductions of infectivity by 20% to 40%) against *Human herpesvirus 1*, *Human adenovirus C* serotype 5, *Influenza A virus*, *Human respiratory syncytial virus*, *Human parainfluenza virus 3*, *Human rhinovirus B* serotype 14, and *Human coxsackievirus* serotype A9. The mechanisms of this antiviral activity are still unclear, but type I interferon induction might be a possible explanation. Further research on this homeopathic preparation seems warranted.

Key words: homeopathy, infections, interferon, cytopathogenic assay, plaque reduction assay.

Résumé : Gripp-Heel[®] est une préparation homéopathique fréquemment utilisée dans le traitement des infections virales respiratoires telles que divers types de grippe ou un rhume banal. On a examiné in vitro l'activité antivirale de Gripp-Heel sur des virus enveloppés et nus à ADN et à ARN pathogènes pour l'humain. Avant de procéder aux essais antiviraux, on a déterminé la cytotoxicité in vitro de Gripp-Heel avec des cellules utilisées pour les expériences d'infection (HeLa, Hep-2, MDCK, BGM) et avec des leucocytes mononucléaires du sang périphérique stimulés par un mitogène. Une concentration de 0,5 d'un produit commercial a légèrement réduit la viabilité et la capacité proliférative des cellules, et les expériences sur la détermination de l'activité antivirale ont débuté avec une dilution de 0,2 du produit. On a déterminé l'activité antivirale contre un grand nombre de virus enveloppés et nus à ADN et à ARN en utilisant la technique de réduction du nombre de plaques, des tests de détermination de l'effet cytopathogène, le titrage des virus, l'analyse des protéines virales dans des dosages immunoenzymatiques spécifiques des virus, et des tests d'hémagglutination. L'acyclovir (10 µg/mL), la ribavirine (6 µg/mL) et le chlorure d'amantadine (5 µg/mL) ont servi de substances témoins selon le type de virus. Gripp-Heel a démontré une activité in vitro dose-dépendante (réductions significatives de l'infectivité de 20 % à 40 %) contre l'herpès virus simplex de type 1, l'adénovirus de type 5, le virus grippal A, le virus respiratoire syncytial, le virus de parainfluenza de type 3, le rhinovirus humain de type 14 et le virus Coxsackie de type 9. Les mécanismes de l'activité antivirale sont toujours inconnus, mais l'induction de l'interféron de type I pourrait fournir une explication. D'autres études sur cette préparation homéopathique sembleraient justifiées.

Mots-clés : homéopathie, infections, interféron, test de détermination de l'effet cytopathogène, technique de réduction du nombre de plaques.

[Traduit par la Rédaction]

Received 15 November 2006. Published on the NRC Research Press Web site at cjpp.nrc.ca on 19 November 2007.

B. Glatthaar-Saalmüller. Labor Dr. Glatthaar, Clinical Immunology, University of Veterinary Medicine Vienna, A-1210 Vienna, Austria (e-mail: glatthaar@1-d-g.de).

¹This article is one of a selection of papers published in this special issue (part 2 of 2) on the Safety and Efficacy of Natural Health Products.

Introduction

The prevalence of respiratory viral infection on a global basis has a significant impact on health care. The most common respiratory viruses include influenza virus, parainfluenza virus, respiratory syncytial virus (RSV), adenovirus, and rhinovirus, each capable of causing respiratory disease, either directly or by exacerbating underlying conditions. Furthermore, many people are latently infected with human

Table 1. Toxicity test for the determination of the in vitro toxicity of Gripp-Heel.

Cells	Relative toxicity, %							
	Serial dilutions of commercially available Gripp-Heel							
	0.5	0.25	0.125	0.063	0.031	0.016	0.0078	0.0039
HeLa	7.51	5.74	4.50	1.85	2.56	3.15	4.14	4.00
Hep-2	11.07	1.12	1.25	-1.29	-4.69	-3.46	-2.74	0.69
MDCK	24.80	2.07	-1.30	-6.86	2.91	-3.84	-3.34	-4.37
BGM	21.52	6.39	2.02	0.57	0.66	-0.85	0.05	6.41
PBMC	40.66	13.05	-0.91	0.63	-1.02	-1.74	2.37	0.39

Note: Viability of HeLa, HEp-2, MDCK (Madin–Darby canine kidney), and BGM cells was analysed by MTT viability tests with varying dilutions of Gripp-Heel to define relative toxicity at day 4. Proliferative capacity of PBMC (peripheral blood mononuclear cells) was quantified after stimulation with Con A (5 µg/mL) and measuring the ³H-thymidine incorporation after 3-day cultivation. Non-treated cells served as control. Their reactivity was determined as 100% reactivity. The influence of Gripp-Heel treatment was quantified according to the method described in Material and methods. Data are means of 3 donors each in 3 replicates.

herpesviruses. In immunocompetent situations, they do not show any clinical symptoms, but herpesviruses are of importance in immunosuppressed individuals and are often responsible for superinfections.

A number of antiviral therapies may be effectively administered to treat respiratory viral disease, thus providing the physician with a range of therapeutic alternatives including amantadine (Hay et al. 1985), neuraminidase inhibitors (Calfee and Hayden 1998), and nucleoside analogues (Fyfe et al. 1978). With many of the therapies available, side effects and systemic toxicity may limit application, particularly in paediatric, geriatric, and immunocompromised patients (Hayden et al. 1983; Englund et al. 1990; Janai et al. 1990). As a result, there is great interest in developing efficacious antiviral compounds that are well tolerated with low toxicity.

Plant-derived substances with detectable antiviral activity in vitro have been described by several research groups (Glatthaar-Saalmüller et al. 2001; Summerfield et al. 1997); however, in vivo data are missing and no information is available about clinical trials with the respective products.

The homeopathic preparation Gripp-Heel® (Biologische Heilmittel Heel GmbH, Baden-Baden, Germany) is frequently used in the treatment of respiratory viral infections such as various types of influenza and the common cold. Gripp-Heel is used in the form of tablets or in solution for injection and contains 5 active ingredients: *Aconitum* (monkshood), *Bryonia* (bryony), *Eupatorium perfoliatum* (water hemp), *Lachesis* (bushmaster snake venom) and phosphorus, all at high dilutions of 10⁻² to 10⁻¹¹. Gripp-Heel is used in complex homeopathic practice, which employs fixed combinations of a number of homeopathic agents.

The objective of our study was to evaluate in vitro the antiviral characteristics of Gripp-Heel in comparison with standard therapeutic antiviral agents.

Materials and methods

Test substance

Commercially available Gripp-Heel solution was supplied free of charge by the manufacturer as sterile ampoules (1.1 mL H₂O) that contained stock solutions of the respective ingredients with declared potencies (D). This refers to

the index of a 10-fold dilution (e.g., D11 indicates a dilution of 10⁻¹¹). The preparation used in the experiments contained *Aconitum* (D4, 4.4 µL), *Bryonia* (D3, 2.2 µL), *Lachesis* (D11, 2.2 µL), *Eupatorium perfoliatum* (D2, 1.1 µL), and phosphorus (D4, 1.1 µL). In all experiments, the test substances were diluted in cell culture medium before addition to the cell cultures.

Reference drugs

Acyclovir (Zovirax, Deutsche Welcome GmbH, Burgwedel, Germany), ribavirin (Virazole, ICN Pharmaceuticals, Frankfurt, Germany), and amantadine (amantadine hydrochloride, Ratiopharm, Ulm, Germany) were used as positive controls. Acyclovir was used in infections with *Human herpesvirus 1* (HHV-1), ribavirin with *Human respiratory syncytial virus*, and amantadine with *Influenza A virus* (Hay et al. 1985; Fyfe et al. 1978; Hruska et al. 1990). These reagents were diluted according to their in vitro cytotoxicity and used in concentrations of 10 µg/mL for acyclovir, 6 µg/mL for ribavirin, and 5 µg/mL for amantadine in the respective in vitro tests.

Cells and viruses

Human rhinovirus B serotype 14 (HRV-14) was obtained from the Institute for Virology of the Friedrich Schiller University, Jena, Germany. RSV, strain Long; *Influenza A virus*, Chile 1/83 (H1N1); *Human parainfluenza virus 3* (Para 3); Human coxsackievirus serotype A9 (CA9); HHV-1, strain Thea; and *Human adenovirus C* serotype 5 (Adeno-5) were obtained from the Department of Medical Virology and Epidemiology of Virus Diseases of the Hygiene Institute of the University of Tübingen, Germany. All viruses were identified and characterized with a panel of monoclonal antibodies (BioWhittaker Products, Walkersville, Md.). HRV was propagated on HeLa cell line, CA9 on BGM, and RSV, Para 3, Adeno-5, and HSV strains on HEp-2, cultured in Earle's minimal essential medium (MEM) with Hanks' buffered saline solution containing 2% foetal calf serum, 25 mmol/L MgCl₂, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. *Influenza A virus* was grown on Madin–Darby canine kidney (MDCK) cells with serum-free MEM containing 1 µg/mL trypsin, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL strepto-

Fig. 1. Relative inhibition of HHV-1 by Gripp-Heel in (a) plaque reduction assay and (b) ELISA with antibodies against viral proteins. Values are means \pm SD, $n = 8$. Asterisks indicate statistical significance versus untreated control at the levels **, $p < 0.01$; ***, $p < 0.001$.

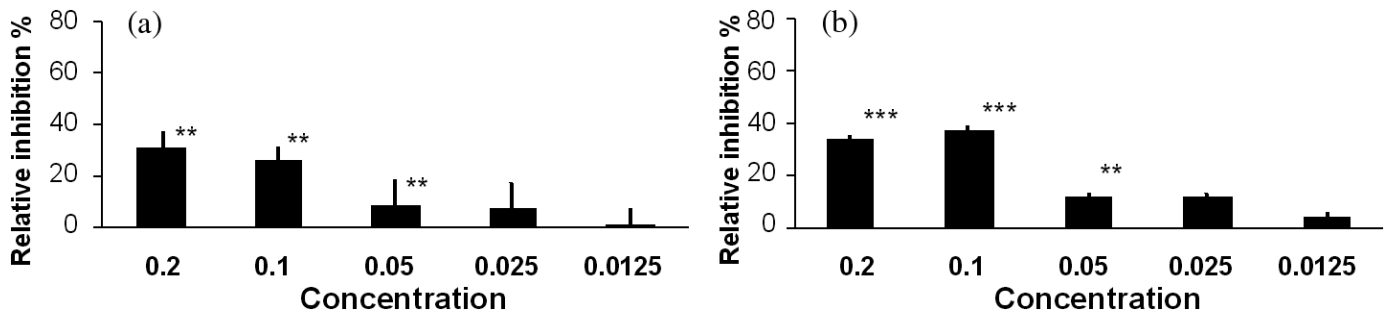


Table 2. Antiviral activity of Gripp-Heel against newly synthesized virus.

Virus	Virus titre, reduction of log ₁₀ TCID ₅₀				
	Gripp-Heel concentrations				
	0.2	0.1	0.05	0.025	0.0125
HHV-1	1.00	0.88	0.75	0.5	-0.12
Adeno-5	0.63	0.63	0.75	0.00	0.00
Influenza A	0.5	0.00	-0.13	-0.25	0.00
RSV	0.5	0.5	0.5	0.63	0.13
Para 3	0.25	0.13	0.13	0.00	0.13
HRV-14	0.38	0.13	0.00	0.25	0.13
CA9	0.75	0.38	0.00	0.38	0.25

Note: TCID₅₀, tissue culture infectious dose. The infectivity of the offspring of Gripp-Heel-treated virus-infected cell cultures were analysed for newly synthesized viruses by virus titration assays and is presented as reduction of log₁₀ TCID₅₀ in relation to the nontreated virus controls.

mycin. To determine the virus titres, the respective cells were incubated in 12- or 24-well tissue culture dishes with serially diluted serum-free virus stock solutions for 1 h at 34 °C. After removal of the virus inoculum, cell cultures were overlaid with the respective virus-specific medium containing agarose or carboxymethylcellulose. Plaques were counted several days later, and the virus titres were calculated as plaque-forming units (PFU) per millilitre.

Virus assays

Enzyme immunoassays, haemagglutination assays, plaque assays, cytopathogenic effects (CPE), and virus titrations were carried out by using standard procedures. For the quantification of antigens of RSV, adenovirus, and herpesvirus, enzyme immunoassays (Virion/Serion, Würzburg, Germany; Merlin Diagnostika GmbH, Bornheim-Hersel, Germany; Dako, Hamburg, Germany) were used. Influenza surface antigens were quantified by haemagglutination assays performed in microplates using chicken erythrocytes. Plaque assays, CPE, and virus titrations for the detection of infectious particles were performed with MDCK, HEp-2, BGM, and HeLa cell cultures.

Cytotoxicity tests

Analyses of the in vitro cytotoxicity of the test substances was measured in 3 different approaches. In a first approach physiologically active cell lines (BGM, HeLa, HEp-2, and MDCK) used in the assays for the detection of antiviral ac-

tivity were analysed for cytotoxicity in an enzymatic assay (MTT assay). This assay enables the quantification of the activity of mitochondrial enzymes in active and dividing cells and shows a direct correlation between viability and enzyme activity (Mosmann 1983; Jackson et al. 1985).

For the determination of the limits of the toxic concentrations of Gripp-Heel, MDCK, HEp-2, BGM, and HeLa cells were cultivated in their growth period together with different dilutions of Gripp-Heel at 37 °C and 5% CO₂ for at least 5 days. Gripp-Heel was therefore diluted in a first step with 10-fold concentrated cell culture medium. Thereafter serial log₂ dilutions were produced and used in the respective cytotoxic assays. The control used in the assay was the respective cell culture media without any component (medium control).

The influence of the test substance on proliferating cells was studied with human peripheral blood mononuclear cells (PBMC) by ³H-thymidine incorporation assays after stimulation with concanavalin A (Con A, 5 µg/mL). PBMC (2 × 10⁵/well) were stimulated with 5 µg/mL Con A for 72 h. Thereafter 1 µCi of ³H-thymidine/well was added to the microcultures for an additional 18 h before the incorporation was stopped by freezing. After harvesting the microcultures on filters, ³H-thymidine incorporation was quantified by liquid scintillation counting. All experiments were performed with PBMC from at least 3 healthy donors in 3 replicates with a serial dilution of the test substance.

Percentage cytotoxicity was calculated with the following formula: [(100 - reactivity of the test group) / (reactivity of the control group)] × 100, where reactivity of the test group was expressed as the optical density (OD) at 450 nm for the MTT test and as counts per minute of ³H-thymidine incorporation for the proliferation assay with PBMC.

Additionally the cytotoxicity of the test substances on the respective cells was monitored by microscopic examination of the cell cultures for altered cell morphology.

Assays for antiviral activity

Plaque-reduction assay and cytopathogenic effects (CPE)

The determination of the antiviral activity of Gripp-Heel against influenza, RSV, Para 3, HRV, CA9, and HHV-1 was performed with plaque-reduction assays (Cooper et al. 1955) or with the analyses of a CPE (Adeno-5). Cell monolayers were infected with a multiplicity of infection (MOI) of 0.0003 (influenza A, CA9), 0.0004 (RSV, HHV-1), 0.0002 (Para 3, HRV), or 0.0006 (Adeno-5) without or in

Fig. 2. Relative inhibition of Adeno-5 by Gripp-Heel in (a) cytopathogenic effect (CPE) assay and (b) ELISA with antibodies against viral proteins. Values are means \pm SD, $n = 8$. Asterisks indicate statistical significance versus untreated control at the levels **, $p < 0.01$; ***, $p < 0.001$.

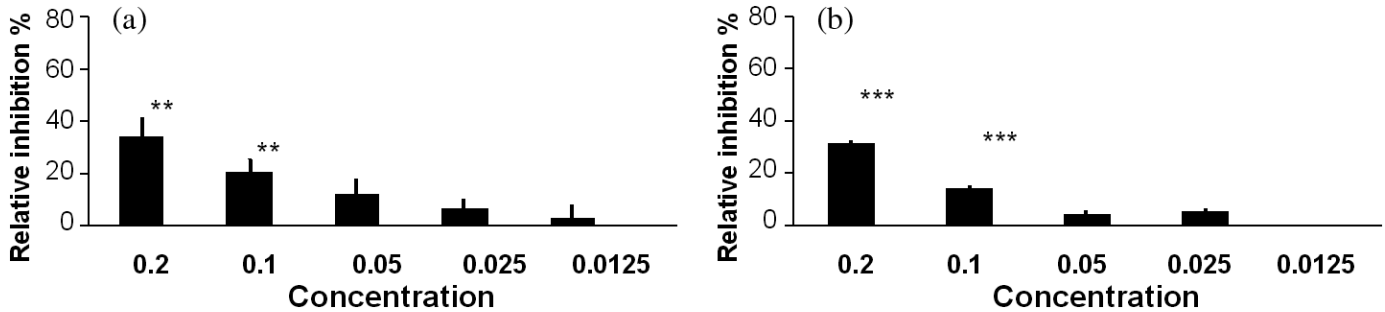


Fig. 3. Relative inhibition of *Influenza A virus* by Gripp-Heel in (a) plaque reduction assay and (b) haemagglutination test. Values are means \pm SD, $n = 8$. Asterisks indicate statistical significance versus untreated control at the levels *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

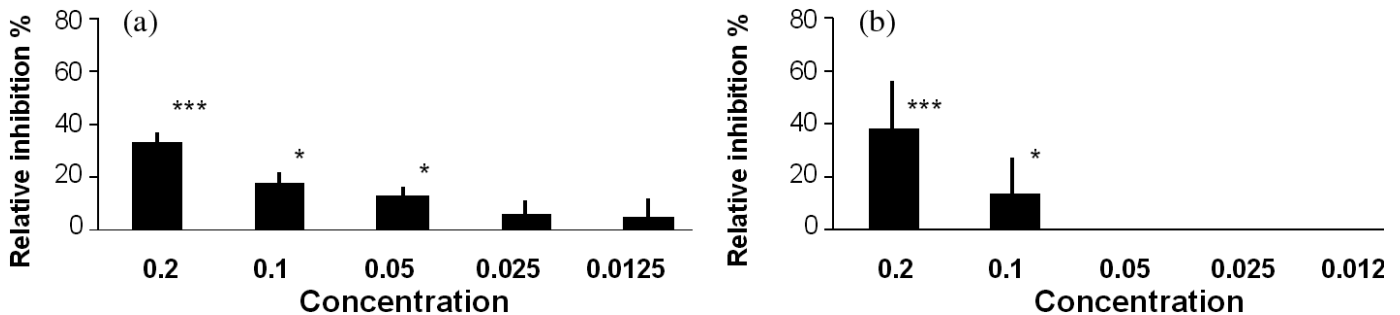
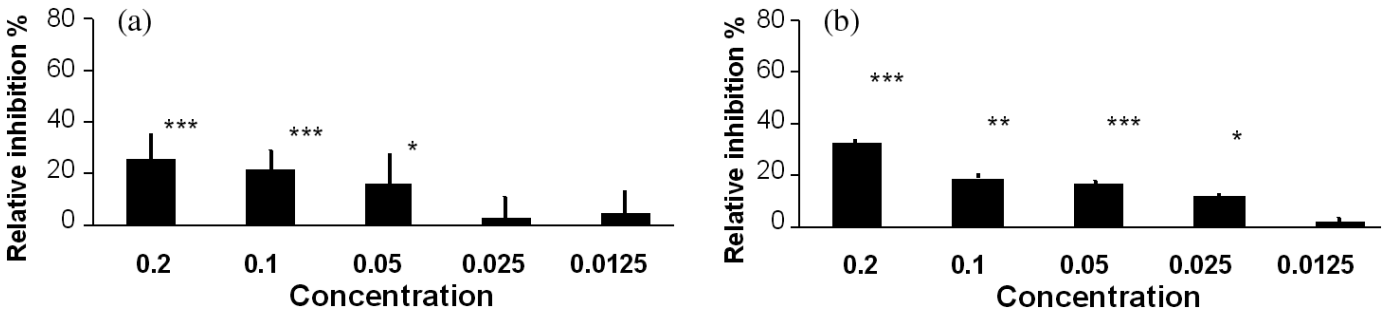


Fig. 4. Relative inhibition of RSV by Gripp-Heel in (a) plaque reduction assay and (b) ELISA with antibodies against viral proteins. Values are means \pm SD, $n = 8$. Asterisks indicate statistical significance versus untreated control at the levels *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.



the presence of different nontoxic dilutions of the test substance and the respective controls. Cells were infected for 1 h at 34 °C. The cell monolayers were then washed and overlaid with medium containing different concentrations of the test substance. Subsequently, the cell cultures were cultivated until lesions were visible in the cell monolayer (plaques or CPE) of the control group cultivated in medium alone. At this time point, the cells were fixed with paraformaldehyde and the remaining cell monolayers stained with a crystal violet solution. Nonstained lesions in the cell monolayer (plaques, CPE) were quantified by employing an optical evaluation system.

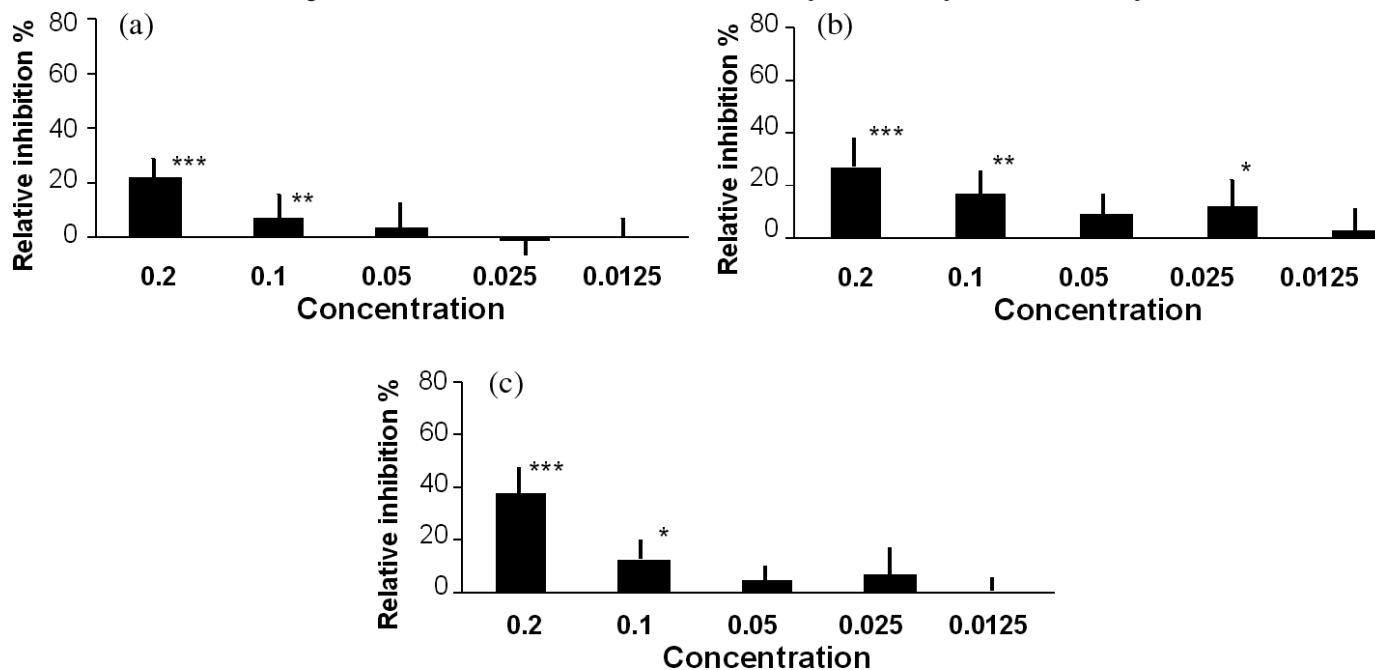
Determination of infectivity of newly synthesized virus preparations

When evaluations of the virus titres of the nontreated con-

trol groups were not possible in plaque-reduction assays mentioned above due to higher infection doses (e.g., high dose MOI 0.02), the determination of the amount of newly synthesized viruses was either performed in ELISA, haemagglutinations assay (HA assay) or by virus titrations on the permissive cell lines, quantified as tissue culture infectious dose (TCID₅₀) values. The quantification of newly synthesized viral proteins was performed with commercially available enzymatic assays according to the manufacturers' specifications. To determinate influenza surface antigens, haemagglutination assays were performed in microtiterplates using chicken erythrocytes (0.5%). Virus-suspensions grown under the influence of the test substance or the control substances were diluted in log₂ steps with PBS before their analyses in the protein assays.

For the determination of the titres (log₁₀ TCID₅₀/mL) of

Fig. 5. Relative inhibition of (a) Para 3, (b) HRV-14 and (c) CA9 by Gripp-Heel in plaque reduction assays. Values are means \pm SD, $n = 8$. Asterisks indicate statistical significance versus untreated control at the levels *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.



virus-suspensions grown under the influence of the test substance, virus-sensitive cell lines were infected with the cell culture supernatants derived from substance-treated cultures and the respective \log_{10} dilutions for 1 h at 34 °C. Thereafter the medium was replaced by semi-solid agarose and the cell cultures were cultivated for an additional 3 to 4 days until lesions in the cell monolayer of the nontreated control groups were visible. The calculation of the respective virus titres (mean infectious dose, \log_{10} TCID₅₀/mL) was done with the Spearman-Kärber method.

Calculation of antiviral activity

The quantification of the antiviral activity, carried out experimentally by plaque-reduction assay (plaque titres), analyses of a CPE, virus-antigen specific ELISA (viral proteins), or influenza surface specific HA tests, is based for all concentrations of the antiviral substances on the mean values of 4 parallel probes and 2 independent tests. The results of the nontreated virus control groups were defined as 100% infection, and in vitro effects of the substances were standardized as relative inhibitory effects.

Results

Control experiments

Commercially available Gripp-Heel solution was tested at different levels of dilution for possible cell toxicity and metabolic modulation effects on the HEP-2, MDCK, HeLa, and BGM cell cultures. Gripp-Heel slightly reduced cell viability within tests at half the concentration of the original solution. No further test substance-related metabolic impairment could be determined at a concentration of 0.25 of the original. Consequently, the preparations were used for evaluations of antiviral activity at a concentration of 0.2 of the

original solution and higher. The results of the mean relative percentage toxicity are presented in Table 1.

Cytotoxic activity of Gripp-Heel against proliferating cells was analysed on human PBMC after stimulation with Con A (5 μ g/mL) in ³H-thymidine incorporation assays with at least PBMC from 3 different healthy donors in triplicate microcultures. The results of the cytotoxicity assays are presented in Table 1.

To verify the test systems, 3 known antiviral substances were used, acyclovir (Zovirax) against HHV-1, amantadine against *Influenza A virus*, and ribavirin (Viracole) against RSV. The effectiveness of the reference substances could be confirmed in the test system. A concentration of 10 μ g/mL acyclovir reduced HHV-1 growth by 74% in a plaque reduction assay and by 77% with ELISA (MOI of 0.0004). Amantadine (5 μ g/mL) reduced *Influenza A virus* plaques by 55% and reduced viral haemagglutinin 50% at an MOI of 0.0003. Ribavirin (6 μ g/mL) produced an approximately 60% reduction in RSV plaques and 64% in ELISA at an MOI of 0.0004.

All control substances reduced the titres of newly synthesized virus quantified as \log_{10} TCID₅₀: with acyclovir (10 μ g/mL) the titre was reduced from 8.0 to 5.75; with amantadine (5 μ g/mL) from 8.0 to 7.25 and with ribavirin (6 μ g/mL) from 6.0 to 4.5 (data not shown).

Antiviral activity of Gripp-Heel on DNA viruses in vitro

The effects of Gripp-Heel were studied on the enveloped DNA virus HHV-1, and on the nonenveloped DNA virus Adeno-5. HHV-1 was studied in HEP-2 cell cultures as described above. Starting with a concentration of 0.2, Gripp-Heel demonstrated a dose-dependent antiviral effect against HHV-1: a reduction of maximally 31% in HHV-1-specific virus plaques was observed (Fig. 1a) and effects of similar

magnitude (33%) were seen on newly synthesized viral proteins in the ELISA (Fig. 1b). The antiviral effect was less with further dilutions. The titre of newly synthesized virus was reduced by Gripp-Heel to a statistically significant extent ($p = 0.0048$): the virus titre (\log_{10} TCID₅₀) with Gripp-Heel at 0.2 concentration was 6.38 compared with 7.38 with the control solution and results in a reduction of one \log_{10} TCID₅₀ (Table 2).

Similar effects were observed on the nonenveloped DNA virus Adeno-5. At the highest concentration of 0.2, Gripp-Heel reduced Adeno-5 infectiousness by 33% in the CPE assay (Fig. 2a), an effect which could be verified with the virus-specific ELISA, where a 32% reduction in viral protein production was observed (Fig. 2b). The antiviral effect was no longer evident at a concentration of 0.05. A significant effect ($p = 0.03$) was seen on titre of newly synthesized virus with a decrease in \log_{10} TCID₅₀ from 5.38 to 4.75 at a Gripp-Heel concentration of 0.2 and results in a reduction of 0.63 \log_{10} TCID₅₀ (Table 2).

Effects of Gripp-Heel on enveloped RNA viruses

Three enveloped RNA viruses were studied: influenza A, RSV, and Para 3.

The effects on *Influenza A virus* were studied in MDCK cell culture. In this study, the test substance at the highest concentration caused a 33% reduction in influenza A-specific virus plaques (Fig. 3a) and a 38% attenuation of the haemagglutinin reaction (Fig. 3b). This effect diminished with the 2 subsequent dilution steps. The titre of newly synthesized virus was significantly lower with Gripp-Heel treatment: virus-titre (\log_{10} TCID₅₀) was 7.25 at the highest concentration of Gripp-Heel versus 7.75 with the control ($p = 0.020$) and results in a reduction of 0.5 \log_{10} TCID₅₀ (Table 2).

Similar effects were seen on RSV in HEp-2 cell culture (Fig. 4a and 4b). Here too, Gripp-Heel at the highest concentration of 0.2 reduced RSV-specific virus infectiousness (by 26% in the plaque reduction assay) and the synthesis of viral protein (33% reduction in ELISA assays). The effects on titre of newly synthesized virus, although significant ($p = 0.020$), were similarly modest as in the experiments with influenza A: the virus titre was 4.88 with the test solution at a concentration of 0.2, compared with 5.38 \log_{10} TCID₅₀ with the control solution and results in a reduction of 0.5 \log_{10} TCID₅₀ (Table 2).

In contrast, the effects of Gripp-Heel on the third enveloped RNA virus, Para 3, analysed using HEp-2 cell cultures, were less prominent. Only the highest concentration showed an effect in plaque reduction assay: a 22% reduction (MOI of 0.0002). No further attenuation effects were shown at greater dilutions (Fig. 5a). There was a nonsignificant reduction ($p = 0.09$) in virus titre with Gripp-Heel at the highest concentration of 0.2 (\log_{10} TCID₅₀ 7.88 versus 8.13 with control, MOI of the inoculate is 0.024, reduction of 0.25 \log_{10} TCID₅₀, Table 2).

Effects on nonenveloped RNA viruses

The effects of Gripp-Heel were assayed on 2 nonenveloped RNA viruses, HRV-14, and CA9.

With HRV-14, inoculated on HeLa cell culture, a relatively modest effect (27%) was observed in the plaque re-

duction assay at the highest concentration of the test solution of 0.2 (MOI 0.0002; Fig. 5b). Virus titre was reduced at the highest titration level of 0.2 (\log_{10} TCID₅₀ with Gripp-Heel: 7.00 versus with control: 7.38; $p = 0.010$, reduction of 0.38 \log_{10} TCID₅₀, Table 2). The MOI of the inoculate was 0.018.

The nonenveloped RNA virus CA9 was inoculated on BGM cell culture. In this study, Gripp-Heel at the 0.2 concentration was associated with a 38% reduction in CA9-specific virus plaques (Fig. 5c). The MOI of the inoculum was 0.0003. The effect was dose dependent and the reduction was smaller with each subsequent dilution step. The effect on newly synthesized virus titre was statistically significant ($p < 0.01$): a reduction of the virus titre from 8.88 with the control to 8.13 (0.75 \log_{10} TCID₅₀) with Gripp-Heel at the 0.2 concentration (MOI 0.018, Table 2).

Discussion

In this *in vitro* study, the homeopathic preparation Gripp-Heel showed inhibitory activity against an exceptionally broad spectrum of virus strains, including enveloped as well as nonenveloped viruses of both DNA and RNA varieties. The antiviral effects were most significant at the highest concentration of 0.2 times the commercially available source solution. The effects were determined in a variety of assays, including plaque reduction assays, virus-specific ELISA, virus titrations assays, microscopic analysis of a CPE and additionally, in the case of influenza A, a haemagglutination test. Dependent upon the virus strain, between 20% and 40% reduction in viral infectiousness was observed. The effects in the plaque reduction assays and ELISA were significant for all viruses tested, whereas in the virus titration assays all virus titres were significantly reduced with the exception of Para 3 where there was a nonsignificant reduction in titre. All antiviral effects were dose-dependent, with lesser impacts observed at greater dilutions.

Gripp-Heel has been used for many decades in Europe and the United States in the treatment of various types of influenza, the common cold and other viral infections. As with most homeopathic medications, there is little research available on the clinical effectiveness and on mechanisms of action of Gripp-Heel. The constituents are commonly used in homeopathic medicine for the indications: inflammatory diseases and cardiac disorders (*Aconitum*), inflammations of respiratory organs and rheumatic disorders (*Bryonia*), influenza, fever, and rheumatic ailments (*Eupatorium*), dermal inflammations (*Lachesis*) and infectious diseases, and vascular disorders (phosphorus) (Rabe et al. 2004).

However, as for most homeopathic preparations, there are few scientifically rigorous studies available. One relatively ambitious observational study in 485 patients by Rabe et al. 2004 indicated that Gripp-Heel has beneficial effects in patients with mild symptoms of viral infections such as fever, headache, muscle pain, cough, nasal congestion, or sore throat. Compared with conventional symptomatic treatments, such as antitussives and nonsteroidal antiinflammatory drugs, the practitioner-rated success rates with treatment were markedly higher with Gripp-Heel than with conventional remedies. The current assay supports the thesis

that the clinical benefits of the homeopathic preparation may be due to direct effects on viral replication. The mechanism behind the effects in the present *in vitro* analyses remain speculative and there is little previous laboratory research to draw on. There was no cell toxicity with Gripp-Heel at the dilutions used in our assays and the effects were not attributable to reduced replication rates of the host cells. Several hypotheses could be advanced, all of which would need testing in specific experimental setups: a direct surface active antiviral reactivity, a direct effect of Gripp-Heel on viral capsid proteins or a direct influence on virus-specific components necessary for viral replication. We recently described the latter effect from a different homeopathic preparation, Engystol (Oberbaum et al. 2005), but as the 2 medications do not share any ingredients, it is quite possible that a different mechanism is responsible for the effects of Gripp-Heel. Gripp-Heel consists of a mixture of highly diluted components. Which of these components are directly responsible for the antiviral effects has not been evaluated. The hypothesis also deserves serious consideration that several mechanisms with possibly additive antiviral effects are involved.

In preliminary experiments (B. Glatthaar-Saalmüller, unpublished data) Gripp-Heel was able to induce type I interferon production in PBMCs after stimulation with inactivated HHV-1. This may suggest a potential mechanism that would also explain the broad reactivity. But it is unknown whether the cells used for virus propagation in the current work are able to produce type I interferons after Gripp-Heel treatment. This will be an important topic for further research.

The effects from Gripp-Heel observed in the current assays were of a slightly smaller magnitude than what was seen with common antiviral drugs: acyclovir, amantadine, and ribavirin. These substances showed high reductions in all antiviral *in vitro* assays. However, these comparisons are only applicable to the conditions of the *in vitro* assays and it is not possible to draw conclusions regarding the relative clinical potencies of the different substances. It should be noted that in clinical use, the antiviral drugs used as controls are all beset by side effects which limit their doses. Gripp-Heel is associated with the good tolerability common to homeopathic medications.

In summary, we have shown that a homeopathic preparation has direct antiviral effects *in vitro* on a range of DNA and RNA viruses. The mechanisms of action of the whole preparation as well as its ingredients need to be studied, and further work on the clinical benefits of this homeopathic preparation would surely be of interest.

References

- Calfee, D.P., and Hayden, F.G. 1998. New approaches to influenza chemotherapy: Neuroaminidase Inhibitors. *Drugs*, **56**: 537–553. doi:10.2165/00003495-199856040-00003. PMID:9806102.
- Cooper, P.D. 1955. A method for producing plaques in agar suspensions of animal cells. *Virology*, **1**: 397–409. doi:10.1016/0042-6822(55)90033-7. PMID:13274741.
- Englund, J.A., Zimmerman, M.E., Swierkosz, E.M., Goodman, J.L., Scholl, D.R., and Balfour, H.H., Jr. 1990. Herpes simplex virus resistant to acyclovir. *Ann. Intern. Med.* **112**: 416–422. PMID:2155570.
- Fyfe, J.A., Keller, P.M., Furman, P.A., Miller, R.L., and Elion, G.B. 1978. Thymidine kinase from Herpes Simplex virus phosphorylates the new antiviral compound 9-(2-hydroxyethoxymethyl) guanine. *J. Biol. Chem.* **253**: 8721–8727. PMID:214430.
- Glatthaar-Saalmüller, B., Sacher, F., and Esperester, A. 2001. Antiviral activity of an extract derived from roots of *Eleutherococcus senticosus*. *Antiviral Res.* **50**: 223–228. doi:10.1016/S0166-3542(01)00143-7. PMID:11397509.
- Hay, A.J., Wolstenholme, A.J., Skehal, J., and Smith, M.H. 1985. The molecular basis of the specific anti-influenza action of amantadine. *EMBO J.* **4**: 3021–3024. PMID:4065098.
- Hayden, F.G., Hoffmann, H.E., and Spyker, D.A. 1983. Differences in side effects of amantadine hydrochloride and rimantadine hydrochloride relate to differences in pharmacokinetics. *Antimicrob. Agents Chemother.* **23**: 458–464. PMID:6847173.
- Hruska, J.F., Bernstein, J.M., Douglas, R.G., and Hall, C.B. 1990. Effects of ribavirin on respiratory syncytial virus *in vitro*. *Antimicrob. Agents Chemother.* **17**: 770–775.
- Jackson, P.R., Pappas, M.G., and Hansen, B.D. 1985. Fluorogenic substrate detection of viable intracellular and extracellular pathogenic protozoa. *Science*, **227**: 435–438. doi:10.1126/science.2578226. PMID:2578226.
- Janai, H.K., Marks, M.I., Zaleska, M., and Stutmanand, H.R. 1990. Ribavirin: Adverse Drug Reactions. *Pediatr. Infect. Dis. J.* **9**: 209–211. PMID:2139930.
- Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**: 55–63. doi:10.1016/0022-1759(83)90303-4. PMID:6606682.
- Oberbaum, M., Glatthaar-Saalmüller, B., Stolt, P., and Weiser, M. 2005. Antiviral activity of Engystol: an *in vitro* analysis. *J. Altern. Complement. Med.* **11**: 855–862. doi:10.1089/acm.2005.11.855. PMID:16296918.
- Rabe, A., Weiser, M., and Klein, P. 2004. Effectiveness and tolerability of a homeopathic remedy compared with conventional therapy for mild viral infections. *Int. J. Clin. Pract.* **58**: 827–832. doi:10.1111/j.1742-1241.2004.00150.x. PMID:15529515.
- Summerfield, A., Keil, G.M., Mettenleiter, T.C., Rziha, H.J., and Saalmüller, A. 1997. Antiviral activity of an extract from leaves of the tropical plant *Acanthospermum hispidum*. *Antiviral Res.* **36**: 55–62. doi:10.1016/S0166-3542(97)00035-1. PMID:9330761.