Anti-HSV-1 and anti-HIV-1 activity of gallic acid and pentyl gallate

Jadel Müller Kratz, Carla Regina Andrighetti-Fröhner/[1]/[3], Deise Juliana Kolling, Paulo César Leal[1], Cláudio César Cirne-Santos[4], Rosendo Augusto Yunes[1], Ricardo José Nunes[1], Edward Trybala[3], Tomas Bergström[3], Izabel CPP Frugulhetti[5], Célia Regina Monte Barardi[2], Cláudia Maria Oliveira Simões/[+]

Laboratório de Virologia Aplicada, Departamento de Ciências Farmacêuticas, [1]Laboratório de Síntese e Estrutura-Atividade, Departamento de Química, [2]Laboratório de Virologia Aplicada, Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Campus Universitário Trindade, 88040-900 Florianópolis, SC, Brasil [3]Departamento de Virologia Clínica, Universidade de Göteborg, Suécia [4]Laboratório de Imunologia Clínica, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brasil [5]Laboratório de Virologia Molecular, Departamento de Biologia Celular e Molecular, Universidade Federal Fluminense, Niterói, RJ, Brasil

The synthetic n-alkyl esters of gallic acid (GA), also known as gallates, especially propyl, octyl and dodecyl gallates, are widely employed as antioxidants by food and pharmaceutical industries. The inhibitory effects of GA and 15 gallates on Herpes Simplex Virus type 1 (HSV-1) and Human Immunodeficiency Virus (HIV-1) replication were investigated here. After a preliminary screening of these compounds, GA and pentyl gallate (PG) seemed to be the most active compounds against HSV-1 replication and their mode of action was characterized through a set of assays, which attempted to localize the step of the viral multiplication cycle where impairment occurred. The detected anti-HSV-1 activity was mediated by the inhibition of virus attachment to and penetration into cells, and by virucidal properties. Furthermore, an anti-HIV-1 activity was also found, to different degrees. In summary, our results suggest that both compounds could be regarded as promising candidates for the development of topical anti-HSV-1 agents, and further studies concerning the anti-HIV-1 activity of this group of molecules are merited.

Key words: antiviral - HSV-1 - HIV-1 - gallic acid - pentyl gallate

Herpes Simplex Virus type 1 (HSV-1) is an enveloped DNA virus that causes one of the most common viral infections in humans, leading to a variety of diseases ranging from mild to severe and sometimes life-threatening (White & Fenner 1994, Whitley & Rozman 2001). Although several nucleoside analogues have been approved for clinical use, such as acyclovir, immunocompromised patients are at increased risk of severity and recurrent infections, since resistant strains have recently been observed (Brady & Bernstein 2004). Therefore, it is desirable to develop new antiviral agents in order to substitute or complement currently available drugs.

The synthetic n-alkyl esters of gallic acid (GA), also known as gallates, especially propyl, octyl and dodecyl gallates, are widely employed as antioxidants by the food and pharmaceutical industries (van der Heijden et al. 1986, Kubo et al. 2002a). Besides the antioxidant activity, other biological activities have been described for this group of molecules, mainly anticancer mechanisms (Fiuza et al. 2004, Kitagawa et al. 2005, Frey et al. 2006, Veluri et al. 2006) as well as antibacterial and antifungal properties (Fujita & Kubo 2002, Kubo et al. 2002b, c, 2003, 2004, Stapleton et al. 2004). However, there are few reports about the antiviral activity of these compounds. In 1988, a study described the inhibition of HSV-1 and HSV-2 replication by methyl gallate (Kane et al. 1988). In 2002, as part of the screening of phenolic compounds against HIV-1 integrase, GA was found to be active (Ahn et al. 2002). More recently, several biological activities of a group of gallates were described by our research group, and various structure-activity relationships regarding their anti-HSV-1, antioxidant and genotoxic effects were proposed (Savi et al. 2005). Furthermore, the pronounced anti-HSV-1 activity of octyl gallate, and its inhibitory effect against RNA viruses were also recently described (Uozaki et al. 2006, Yamasaki et al. 2007).

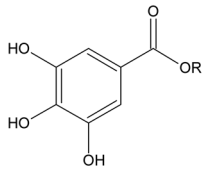
In the present study, GA and 15 gallates were re-evaluated for anti-HSV-1 and anti-HIV-1 activities, followed by selection of the most active anti-HSV-1 compounds and the determination of the viral multiplication step(s) upon which these compounds act.

MATERIALS AND METHODS

Compounds - GA and 15 gallates (with increasing number of carbons in the alkyl chain; Fig. 1) were synthesized as previously described (Savi et al. 2005). The compounds (50 mM) were dissolved in dimethyl sulphoxide, stored at -20°C protected from light, and further diluted in culture medium prior to use. Acyclovir was purchased from Sigma (St. Louis, USA).

Cells and viruses - For the anti HSV-1 activity screening, Vero cells were grown in minimum essential medium (MEM; Cultilab, São Paulo, Brazil) supplemented with 10% fetal bovine serum (FBS; Cultilab, São Paulo, Brazil), penicillin (100 U/ml), streptomycin (100 µg/ml)

Financial support: CNPq/MCT/Brazil (proc n. 472748/2004-1), Capes
+ Corresponding author: claudias@reitoria.ufsc.br
Received 22 January 2008
Accepted 26 June 2008



Radical	Compound	CC_{50} [a]	IC_{50} [b]	SI[c]
H	Gallic acid	668.7 ± 54.5	57.1 ± 2.3	11.72
CH_3	Methyl gallate	> 1,000	102.4 ± 2.7	> 9.76
C_2H_5	Ethyl gallate	893.9 ± 107.2	112.5 ± 19.7	7.94
C_3H_7	Propyl gallate	713.2 ± 153.1	110.8 ± 20.7	6.44
C_4H_9	Butyl gallate	361.6 ± 92.8	101.5 ± 26.0	3.56
C_5H_{11}	Pentyl gallate	268.7 ± 45.2	45.4 ± 2.5	6.08
-	Acyclovir	> 1,000	0.43 ± 0.14	> 2,325

Fig. 1: chemical structures of gallic acid and some of the n-alkyl esters (gallates) evaluated. The cytotoxicity was determined by MTT assay and the anti-HSV-1 activity by viral plaque number reduction assay. Acyclovir was used as a positive control. The mean values ± standard deviations are representative of three independent experiments. CC_{50}: 50% cytotoxic concentration, defined as the concentration (μM) that reduced cell viability by 50% when compared to untreated controls; IC_{50}: 50% inhibitory concentration, defined as the concentration (μM) that inhibited 50% of viral plaque formation when compared to untreated controls; SI: selectivity index is the ratio between CC_{50} and IC_{50} values.

and amphotericin B (25 μg/ml; Cultilab, São Paulo, Brazil). Cell cultures were maintained at 37°C in a humidified 5% CO_2 atmosphere. The HSV-1 strain KOS (Faculty of Pharmacy, University of Rennes, France) was propagated in Vero cells, while stock viruses were prepared as previously described (Simões et al. 1999). After three cycles of freezing/thawing, the fluids were titrated on the basis of PFU count as previously described (Burleson et al. 1992) and stored at -80°C until use. Purified extracellular HSV-1 particles were obtained as previously described (Karger & Mettenleiter 1993), with minor modifications. The specific activity was 1.29×10^{-2} (counts per minute [cpm]/PFU).

For the anti-HIV-1 activity screening, peripheral blood mononuclear cells (PBMCs) from healthy human donors were obtained by density gradient centrifugation (Histopaque; Sigma, St. Louis, USA) from buffy coat preparations. PBMCs were resuspended in RPMI 1640 (LGC Bio, São Paulo, Brazil) supplemented with 10% FBS (Hyclone, Logan, USA), penicillin and streptomycin (Cultilab, São Paulo, Brazil), 2 mM glutamine and 10 mM HEPES (Sigma, St. Louis, USA), stimulated with 5 μg/ml of phytohemagglutinin (PHA; Sigma, St. Louis, USA) for two to three days, and further maintained in culture medium containing 5 U/ml of recombinant human interleukin-2 (Sigma, St. Louis, USA). HIV-1 isolate Ba-L (R5-tropic, subtype B; Cirne-Santos et al. 2008) was used to infect cells. Virus isolates were prepared in PHA-activated PBMCs from human healthy donors.

Cytotoxicity evaluation - Confluent cells were exposed to different concentrations of compounds for 72 h. After incubation, cell viability was assessed by a MTT [3-(4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide] assay (Mosmann 1983). The 50% cytotoxic concentration (CC_{50}) was defined as the concentration (μM) that reduced cell viability by 50% when compared to untreated controls.

Screening of in vitro anti-HSV-1 activity - Confluent Vero cells were infected with HSV-1 at a MOI of 0.5 and treated with non-cytotoxic concentrations of each compound for 72 h. Acyclovir (10 μM) was used as a positive control. The same method used to evaluate cell viability with MTT was followed with appropriate modifications (Takeuchi et al. 1991), and the 50% inhibitory concentration (IC_{50}) was defined as the concentration (μM) that reduced the absorbance of infected cells to 50% when compared to untreated controls.

Viral plaque number reduction assay - This assay followed the procedures previously described (Kuo et al. 2001) with minor modifications. Acyclovir (10 μM) was used as a positive control. Approximately 100 PFU of HSV-1 were adsorbed for 1 h at 37°C on confluent Vero cells. Cultures were then washed twice with PBS and overlaid with MEM containing 1.5% carboxymethylcellulose and different concentrations of compounds. After 72 h, cells were fixed and stained with naphtol blue-black and plaques were counted. The IC_{50} was defined as the concentration (μM) that inhibited 50% of viral plaque formation when compared to untreated controls.

Virucidal assay - This assay followed procedures that have been previously described (Ekblad et al. 2006). Mixtures of GA or pentyl gallate (PG) and 4.0×10^4 PFU of HSV-1 in serum-free MEM were co-incubated for 20 min at 37°C in a water bath prior to the dilution of the mixture to non-inhibitory concentrations of compound (1:100), and the residual infectivity was determined by a viral plaque number reduction assay, as described above.

Attachment and penetration assays - The attachment assay followed procedures that have been described previously (Ekblad et al. 2006) with minor modifications. Different concentrations of GA or PG were mixed with purified radiolabelled HSV-1 and incubated for 15 min at 37°C. Then, the virus-compound mixtures were adsorbed for 2 h at 4°C on confluent cells. Subsequently, cultures were washed twice with PBS supplemented with 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (PBS-A) and lysed with PBS-A containing 5% sodium dodecyl sulphate (SDS), after which the lysates were transferred to scintillation vials for radioactivity quantification. The penetration assay followed the procedures previously described (Cheng et al. 2004) with minor modifications. Approximately 100 PFU of HSV-1 were adsorbed for 2 h at 4°C on confluent cells, washed twice with PBS and incubated in the presence or absence of different concentrations of GA or PG at 37°C to maximize the penetration of viruses. After 10 min, cultures were treated for 1 min with warm citrate-buffered saline (pH 3) to inactivate the unpenetrated viruses and the inhibitory activity of the compounds was determined by a viral plaque number reduction assay.

Western blotting analysis - This assay followed the procedures described previously (Kuo et al. 2001) with

minor modifications. Confluent cells were infected with HSV-1 at a MOI of 0.1 and after 1 h of adsorption at 37°C, cultures were incubated in the presence or absence of 125 μM of GA or PG. At 18 h post-incubation, cellular proteins were extracted, dissolved (~50 μg; determined by the Bradford procedure) in the dissociation buffer (2% SDS, 5% β-mercaptoethanol, 0.125 M Tris-HCl, 30% glycerol, 0.8% bromophenol blue, and 100 μg/ml phenylmethylsulfonyl fluoride; Sigma, St. Louis, USA) and boiled for 5 min. The proteins were then resolved by 10% SDS-PAGE and transferred to Immobilon PVDF® membranes (Millipore, Billerica, USA). Membranes were blocked overnight with 10% nonfat dry milk in Tris-Base saline buffer, and then incubated with the following antibodies: mouse monoclonal antibodies raised against HSV-1 γ proteins gD (Santa Cruz Biotechnology, Santa Cruz, USA) and gC (Sjogren-Jansson & Jeansson 1985, 1990); rabbit polyclonal antibody raised against HSV-1 γ protein VP5 (kindly provided by G Cohen and R Eisenberg, University of Pennsylvania, Philadelphia); and goat polyclonal antibody raised against HSV-1 α protein ICP27 (Santa Cruz Biotechnology, Santa Cruz, USA). Specific reactive proteins were detected by a diaminobenzidine (DAB) colorimetric reaction, after incubation with rabbit anti-mouse, swine anti-rabbit or donkey anti-goat immunoglobulin secondary antibodies, respectively, linked to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, USA).

Drug combination assay - This assay followed procedures that have been previously described (Gong et al. 2004) with minor modifications. A viral plaque number reduction assay was performed as described above, and the treatment consisted of GA, PG or acyclovir alone (0.125 x IC_{50}, 0.25 x IC_{50}, 0.5 x IC_{50}, 1 x IC_{50}, 2 x IC_{50}), and a mixture of varying concentrations of each compound in a fixed ratio (i.e., 0.5 x IC_{50} of GA + 0.5 x IC_{50} of acyclovir; 0.25 x IC_{50} of GA + 0.25 x IC_{50} of PG). Based on inhibitory activities, the synergistic or antagonistic antiviral effect among compounds was determined using the software MacSynergy II (kindly provided by Mark Prichard, University of Michigan, USA), through a three-dimensional analytical method (Prichard et al. 1993).

Screening of in vitro anti-HIV-1 activity - This assay followed the procedures described previously (Cirne-Santos et al. 2008) with minor modifications. Peripheral blood mononuclear cells (PBMCs) were initially exposed to viral suspensions containing 5-10 ng/ml of HIV-1 p24 Ag during 2-3 h. Cells were washed, resuspended in complete medium, plated in 96-well culture plates (2.0 x 10^5 cells/well) in triplicate, and treated with 50 μM of GA and gallates. After seven days at 37°C in 5% CO_2, viral replication was assessed by measuring the HIV-1 p24 Ag presence in culture supernatants by an ELISA capture assay (ZeptoMetrix Co., Buffalo, USA).

Statistical analysis - The mean values ± standard deviations are representative of three independent experiments. For the determination of CC_{50} and IC_{50} values, non-linear regressions of concentration-response curves were used.

RESULTS

Cytotoxicity evaluation and anti-HSV-1 activity - GA and 15 gallates were evaluated for their cytotoxicity and anti-HSV-1 activity in Vero cells by the MTT assay. Considering the selectivity indices (SI) obtained in this preliminary screening, the most active compounds were: GA (39.1), methyl (3.5), ethyl (6.3), propyl (5.1), butyl (5.4) and pentyl (9.2) gallates. These compounds were selected to have their antiviral activity confirmed by a viral plaque number reduction assay. For these purposes, acyclovir was used as a positive control. The results of the antiviral activity confirmation are summarized in Fig. 1. As shown, GA and PG presented lower IC_{50} values among the compounds evaluated and at the highest concentrations tested (125 μM), only these two compounds thoroughly inhibited HSV-1 replication. Thus, the following study was focused on these compounds.

Effect of GA and PG on HSV-1 attachment and penetration - To elucidate whether the anti-HSV-1 activity of GA and PG was related to the blockage of the early events of viral infection, their effects on viral attachment to and penetration into cells were investigated. Fig. 2 shows the observed results, for which both compounds were active: GA presented a greater effect in the attachment assay (Fig. 2A), with an IC_{50} value of 23.9 ± 9.4 μM, and PG was more active in the penetration assay (Fig. 2B), with an IC_{50} value of 9.1 ± 3.2 μM. Furthermore, in order to identify if the cells or the virus particles were affected, cells were pre-treated (3 h) with compounds. The compounds were then washed out, and thereafter cells were infected with 100 PFU of HSV-1. The results showed that the pre-treatment of cells with both compounds did not reduce HSV-1 infectivity (data not shown), suggesting that the virus particles were targeted.

Virucidal activity of GA and PG - The virus-inactivating activity of these compounds in the absence of cells was evaluated (Fig. 3). The co-incubation of 4.0 x 10^4 PFU of HSV-1 with different concentrations of compounds followed by the dilution of the mixture to non-inhibitory concentrations revealed that both GA and PG caused complete inactivation of HSV-1 infectivity at relatively low IC_{50} values.

Effect of GA and PG on HSV-1 protein synthesis - We analyzed whether GA and PG inhibition of HSV-1 replication was related to the blockage of viral protein synthesis. Accordingly, the expression of ICP27, gC, gD and VP5 viral proteins in infected Vero cells was also evaluated. As shown in Fig. 4, while uninfected cells (Lane 1) did not express these proteins, all were detectable in HSV-1 infected cells (Lane 2). Treatment with GA (Lane 3) and PG (Lane 4) suppressed the expression of these proteins. These results showed that both GA and PG inhibited HSV-1 replication when cells were already infected.

Effect of GA and PG in combination with acyclovir - The treatment of infected cells with a mixture of GA or PG and acyclovir revealed that neither a synergistic nor an antagonistic antiviral effect among compounds was observed (data not shown).

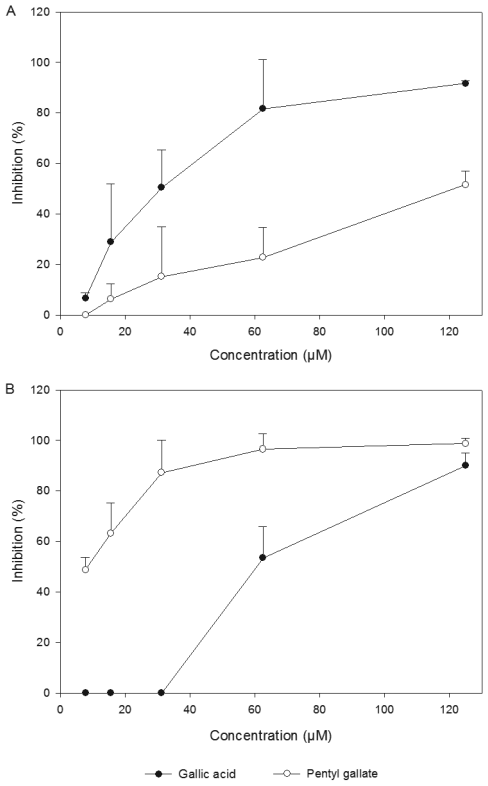


Fig. 2: effect of gallic acid and pentyl gallate on HSV-1 attachment to (A) and penetration into cells (B). A: mixtures of each compound and purified radiolabelled HSV-1 were incubated for 15 min at 37°C prior to the addition to cells. After virus adsorption for 2h at 4°C, cells were washed and then lysed with 5% SDS to quantify radioactivity; B: approximately 100 PFU of HSV-1 were adsorbed for 2h at 4°C on cells and incubated in the presence or absence of different concentrations of GA or PG at 37°C to maximize the penetration of viruses. After 10 min, cultures were treated for 1 min with warm citrate-buffered saline (pH 3) to inactivate the unpenetrated viruses. The mean values ± standard deviations are representative of three independent experiments.

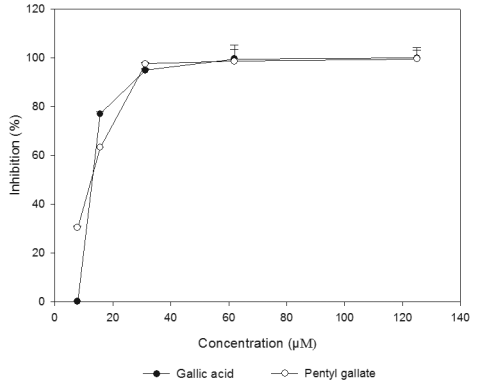


Fig. 3: virus-inactivating activity of gallic acid and pentyl gallate determined by viral plaque number reduction assay. Different concentrations of both compounds were incubated with 4.0 x 10^4 PFU of HSV-1 for 20 min at 37°C followed by the dilution of the mixtures to non-inhibitory concentrations prior to their addition to cells. The mean values ± standard deviations are representative of three independent experiments.

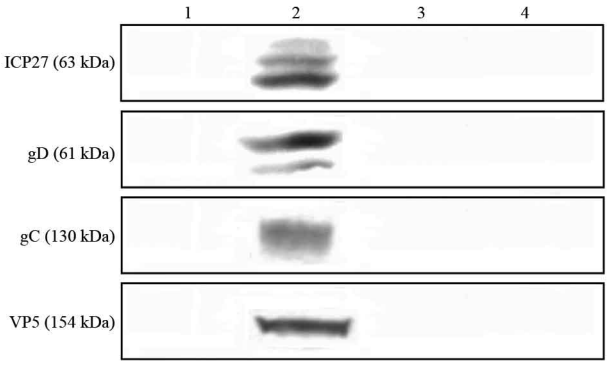


Fig. 4: effect of gallic acid (GA) and pentyl gallate (PG) on HSV-1 protein expression in Vero cells detected by Western blotting analysis. Confluent Vero cells were infected with HSV-1 (MOI 0.1) and treated with GA or PG. Lysates (~50 µg of protein) were collected at 18h post-incubation and run on SDS-10% PAGE gel and analyzed by immunoblotting with anti-ICP27, gD, gC, VP5 antibodies. Lanes - 1: uninfected Vero cells; 2: HSV-1 infected Vero cells; 3: HSV-1 infected Vero cells treated with GA; 4: HSV-1 infected Vero cells treated with PG.

Effect of GA and other gallates on HIV-1 multiplication - In addition, GA and 15 gallates were screened for anti-HIV-1 activity. As shown in Table, the higher percentages of inhibition were found to be among the compounds with no more than five carbons in the alkyl moiety. A similar association between the size of the alkyl moiety and the antiviral activity was found for HSV-1.

DISCUSSION

In a previous study, our research group described some biological effects of GA and its alkyl esters (gallates). Although structure-activity relationships regarding the anti-HSV-1, antioxidant, and genotoxic effects were proposed, the steps of viral replication affected by these compounds were not determined (Savi et al. 2005). In this study, GA and 15 gallates were evaluated for their in vitro anti-HSV-1 activity by a more robust methodology (viral plaque assay), and the best candidates had their mode of action studied. The screening process started with the evaluation of cytotoxic effects of these compounds in Vero cells followed by the determination of their antiviral activity by MTT assay. GA and gallates with as much as five carbons in the alkyl chain presented the optimum anti-HSV-1 activity (higher SI values) among the evaluated compounds, and therefore, their anti-HSV-1 activity was confirmed by a viral plaque number reduction assay (Fig. 1), in which GA and PG were chosen due to their higher percentages of HSV-1 inhibition.

Therefore, the anti-HSV-1 activity of GA and PG was scrutinized. The combination of GA and PG with acyclovir resulted in no interaction (data not shown). The pretreatment of cells with GA and PG did not affect viral infectivity (data not shown). Thus, neither GA nor PG affects the viral infection process through their binding to cell membrane molecules. However, the addition of compounds concomitantly with the virus, at specific conditions, revealed that both compounds affect virus attachment to and penetration into cells (Fig. 2). These observations suggest

TABLE

Effect of gallic acid (GA) and gallates on HIV-1 multiplication. Peripheral blood mononuclear cells (PBMCs) were exposed to viral suspensions containing 5 to 10 ng/ml of HIV-1 p24 Ag. Then, cells were washed, plated and treated with 50 μM of GA and gallates. After seven days, viral replication was assessed by measuring the presence of HIV-1 p24 Ag in culture supernatants by an ELISA capture assay

Radical	Compound	CC_{50} [a]	%[b]
H	Gallic acid	825	89
CH_3	Methyl gallate	1230	40
C_2H_5	Ethyl gallate	2100	48
C_3H_7	Propyl gallate	1800	95
C_4H_9	Butyl gallate	1460	90
C_5H_{11}	Pentyl gallate	1525	78
C_6H_{13}	Hexyl gallate	1580	35
C_7H_{15}	Heptyl gallate	1718	48
C_8H_{17}	Octyl gallate	1835	53
C_9H_{19}	Nonyl gallate	2120	38
$C_{10}H_{21}$	Decyl gallate	2335	45
$C_{11}H_{23}$	Undecyl gallate	2048	48
$C_{12}H_{25}$	Dodecyl gallate	2200	40
$C_{14}H_{29}$	Tetradecyl gallate	1800	51
$C_{16}H_{33}$	Hexadecyl gallate	1720	44
$C_{18}H_{37}$	Octadecyl gallate	1145	33

a: the 50% cytotoxic concentration (CC_{50}) was defined as the concentration (μM) that reduced cell viability by 50% when compared to untreated controls by MTT assay; *b*: percentages of viral replication inhibition when compared to untreated controls.

that GA and PG affect HSV-1 infectivity possibly by detaching viruses that have already bound to cells, perhaps through the disturbance of viral glycoproteins.

In order to verify these findings, the virucidal activity of compounds was evaluated. After 20 min of co-incubation at 37°C, both compounds caused complete inactivation of HSV-1 in the absence of cells at low IC_{50} values (Fig. 3). The direct virucidal activity was also found for other gallate derivatives, such as epigallocatechin-3-gallate and octyl gallate (Song et al. 2005, Uozaki et al. 2006).

Subsequently, we examined the effects of both compounds when the viruses have already penetrated into cells and started their replication. The results showed that the expression of ICP27, gC, gD, and VP5 proteins was inhibited in infected cells treated with GA and PG (Fig. 4). To clarify these results and eliminate any possibility of cytotoxicity of compounds on infected cells, the viability of infected Vero cells treated with GA and PG was evaluated by a trypan blue dye exclusion method. The results showed that viability was not substantially affected when compared to untreated infected controls (data not shown).

These results suggested that the inhibition of the expression of viral proteins by GA and PG may be attributed to their virucidal effect on the new progenies of virus, avoiding the cell-to-cell spread of infection.

During the course of our studies, Uozaki et al. (2006) described anti-HSV-1 activity of octyl gallate with a moderate cytotoxicity. In our study, the cytotoxicity presented a similar profile, although the antiviral activity was found only for the gallates with as much as seven carbons in the alkyl moiety. The discrepancies between the results of these studies may come from the different cell lines employed and methodologies used, as already supposed by the authors (Uozaki et al. 2006).

The anti-HIV-1 activity of GA and gallates was also screened (Table I). The cytotoxicity of GA and gallates on PBMCs did not present a clear concentration-response pattern, with CC_{50} values higher then 1,000 μM (except GA), while the cytotoxic effects on Vero cells were superior and increased along with the number of carbons in the alkyl moiety, reaching a maximum effect at the compound with 11 carbons (undecyl gallate; data not shown). In relation to anti-HIV-1 activity, the results showed that GA and gallates with no more than five carbons in the alkyl moiety presented higher percentages of viral replication inhibition (≥ 78%). A similar association between the size of the alkyl moiety and the antiviral activity was found for HSV-1, suggesting that the lipophilicity of these molecules may be involved in their biological properties, as already suggested (Rosso et al. 2006).

In summary, the anti-HSV-1 activity of GA and PG was attributed to the inhibition of virus attachment to and penetration into cells, as well as to their virucidal effects. These results suggest that both compounds could be regarded as promising candidates for the development of topical anti-HSV-1 agents, and further studies concerning the anti-HIV-1 activity of this group of molecules are merited.

ACKNOWLEDGEMENTS

To the Hemotherapy Service of the Hospital Clementino Fraga Filho (UFRJ), for providing buffy coats.

REFERENCES

Ahn MJ, Kim CY, Lee JS, Kim TG, Kim SH, Lee CK, Lee BB, Shin CG, Huh H, Kim J 2002. Inhibition of HIV-1 integrase by galloyl glucoses from *Terminalia chebula* and flavonol glycoside gallates from *Euphorbia pekinensis*. *Planta Med 68*: 457-459.

Brady RC, Bernstein DJ 2004. Treatment of herpes simplex virus infections. *Antiviral Res 61*: 73-81.

Burleson FG, Chamberts TM, Wiedbrauk DL 1992. *Virology*: *a laboratory manual*, Academic Press, San Diego, 250 pp.

Cheng HY, Lin TC, Yang CM, Wang KC, Lin LT, Lin CC 2004. Putranjivain A from *Euphorbia jolkini* inhibits both virus entry and late stage replication of herpes simplex virus type 2 in vitro. *J Antimicrob Chemother 53*: 577-583.

Cirne-Santos CC, Souza TM, Teixeira VL, Fontes CF, Rebello MA, Castello-Branco LR, Abreu CM, Tanuri A, Frugulhetti IC, Bou-Habid DC 2008. The dolabellane diterpene Dolabelladienetriol is a typical noncompetitive inhibitor of HIV-1 reverse transcriptase enzyme. *Antiviral Res 77*: 64-71.

Ekblad M, Bergström T, Banwell MG, Bonnet M, Renner J, Ferro V, Trybala E 2006. Anti-herpes simplex virus activities of two novel disulphated cyclitols. *Antivir Chem Chemother 17*: 97-106.

Fiuza SM, Gomes C, Teixeira LJ, Girão da Cruz MT, Cordeiro MN, Milhazes N, Borges F, Marques MP 2004. Phenolic acid deriva-

tives with potential anticancer properties - a structure-activity relationship study. Part 1: methyl, propyl and octyl esters of caffeic and gallic acids. *Bioorg Med Chem 12*: 3581-3589.

Frey C, Pavani M, Cordano G, Muñoz S, Rivera E, Medina J, Morello A, Diego Maya J, Ferreira J 2006. Comparative cytotoxicity of alkyl gallates on mouse tumor cell lines and isolated rat hepatocytes. *Comp Biochem Physiol A Mol Integr Physiol 146*: 520-527.

Fujita K, Kubo I 2002. Antifungal activity of octyl gallate. *Int J Food Microbiol 79*: 193-201.

Gong Y, Raj KM, Luscombe CA, Gadawski I, Tam T, Chu J, Gibson D, Carlson R, Sacks SL 2004. The synergistic effects of betulin with acyclovir against herpes simplex viruses. *Antiviral Res 64*: 127-130.

Kane CJ, Menna JH, Sung CC, Yeh YC 1988. Methyl gallate, methyl-3,4,5-trihydoxybenzoate, is a potent and highly specific inhibitor of herpes simplex virus in vitro. II. Antiviral activity of methyl gallate and its derivatives. *Biosci Rep 8*: 95-102.

Karger A, Mettenleiter TC 1993. Glycoproteins gIII and gp50 play dominant roles in the biphasic attachment of pseudorabies virus. *Virology 194*: 654-664.

Kitagawa S, Nabekura T, Kamiyama S, Takahashi T, Nakamura Y, Kashiwada Y, Ikeshiro Y 2005. Effects of alkyl gallates on P-glycoprotein function. *Biochem Pharmacol 70*: 1262-1266.

Kubo I, Fujita K, Nihei K 2002a. Anti-*Salmonella* activity of alkyl gallates. *J Agric Food Chem 50*: 6692-6696.

Kubo I, Fujita K, Nihei K, Masuoka N 2003. Non-antibiotic antibacterial activity of dodecyl gallate. *Bioorg Med Chem 11*: 573-580.

Kubo I, Fujita K, Nihei K, Nihei A 2004. Antibacterial activity of alkyl gallates against *Bacillus subtilis*. *J Agric Food Chem 52*: 1072-1076.

Kubo I, Masuoka N, Xiao P, Haraguchi H 2002b. Antioxidant activity of dodecyl gallate. *J Agric Food Chem 50*: 3533-3539.

Kubo I, Xiao P, Fujita K 2002c. Anti-MRSA activity of alkyl gallates. *Bioorg Med Chem Lett 12*: 113-116.

Kuo YC, Chen CC, Tsai WJ, Ho YH 2001. Regulation of herpes simplex virus type 1 replication in Vero cells by *Psychotria serpens*: relationship to gene expression, DNA replication, and protein synthesis. *Antiviral Res 51*: 95-109.

Mosmann T 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods 65*: 55-63.

Prichard MN, Prichard LE, Shipman C 1993. Strategic design and three-dimensional analysis of antiviral drug combinations. *Antimicrob Agents Chemother 37*: 540-545.

Rosso R, Vieira TO, Leal PC, Nunes RJ, Yunes RA, Creczynski-Pasa TB 2006. Relationship between the lipophilicity of gallic acid n-alquil esters' derivatives and both myeloperoxidase activity and HOCl scavenging. *Bioorg Med Chem 14*: 6409-6413.

Savi LA, Leal PC, Vieira TO, Rosso R, Nunes RJ, Yunes RA, Creczynski-Pasa TB, Barardi CR, Simões CM 2005. Evaluation of anti-herpetic and antioxidant activities, and cytotoxic and genotoxic effects of synthetic alkyl-esters of gallic acid. *Arzneimittelforschung 55*: 66-75.

Simões CM, Amoros M, Girre L 1999. Mechanism of antiviral activity of triterpenoid saponins. *Phytother Res 13*: 323-328.

Sjogren-Jansson E, Jeansson S 1985. Large-scale production of monoclonal antibodies in dialysis tubing. *J Immunol Methods 84*: 359-364.

Sjogren-Jansson E, Jeansson S 1990. Growing hybridomas in dialysis tubing: optimization of the technique. In: H Zola, *Laboratory methods in immunology*, CRC Press, Boca Raton, p. 41-50.

Song JM, Lee KH, Seong BL 2005. Antiviral effect of catechins in green tea on influenza virus. *Antiviral Res 68*: 66-74.

Stapleton PD, Shah S, Anderson JC, Hara Y, Hamilton-Miller JM, Taylor PW 2004. Modulation of beta-lactam resistance in *Staphylococcus aureus* by catechins and gallates. *Int J Antimicrob Agents 23*: 462-467.

Takeuchi H, Baba M, Shigeta S 1991. An application of tetrazolium (MTT) colorimetric assay for the screening of anti-herpes simplex virus compounds. *J Virol Methods 33*: 61-71.

Uozaki M, Yamasaki H, Katsuyama Y, Higuchi M, Higuti T, Koyama AH 2006. Antiviral effect of octyl gallate against DNA and RNA viruses. *Antiviral Res 73*: 85-91.

van der Heijden CA, Janssen PJ, Strik JJ 1986. Toxicology of gallates: a review and evaluation. *Food Chem Toxicol 24*: 1067-1070.

Veluri R, Singh RP, Liu Z, Thompson JA, Agarwal R, Agarwal C 2006. Fractionation of grape seed extract and identification of gallic acid as one of the major active constituents causing growth inhibition and apoptotic death of DU145 human prostate carcinoma cells. *Carcinogenesis 27*: 1445-1453.

White DO, Fenner FJ 1994. *Medical virology*, Academic Press, San Diego, 603 pp.

Whitley RJ, Roizman B 2001. Herpes simplex virus infections. *Lancet 357*: 1513-1518.

Yamasaki H, Uozaki M, Katsuyama Y, Utsunomiya H, Arakawa T, Higuchi M, Higuti T, Koyama AH 2007. Antiviral effect of octyl gallate against influenza and other RNA viruses. *Int J Mol Med 19*: 685-688.