Original article

Antiviral activity of lauryl gallate against animal viruses

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Background: Antiviral compounds are needed in the control of many animal and human diseases.
Methods: We analysed the effect of the antitumoural drug lauryl gallate on the infectivity of the African swine fever virus among other DNA (herpes simplex and vaccinia) and RNA (influenza, porcine transmissible gastroenteritis and Sindbis) viruses, paying attention to its effect on the viability of the corresponding host cells.
Results: Viral production was strongly inhibited in different cell lines at non-toxic concentrations of the drug (1–10 µM), reducing the titres 3–5 log units depending on the multiplicity of infection. In our model system (African swine fever virus in Vero cells), the addition of the drug 1 h before virus adsorption completely abolished virus productivity in a one-step growth virus cycle. Interestingly, no inhibitory effect was observed when lauryl gallate was added after 5–8 h post-infection. Both cellular and viral DNA synthesis and late viral transcription were inhibited by the drug; however, the early viral protein synthesis and the virus-mediated increase of p53 remained unaffected. Activation of the apoptotic effector caspase-3 was not detected after lauryl gallate treatment of Vero cells. Furthermore, the presence of the drug abrogated the activation of this protease induced by the virus infection.
Conclusions: Lauryl gallate is a powerful antiviral agent against several pathogens of clinical and veterinary importance. The overall results indicate that a cellular factor or function might be the target of the antiviral action of alkyl gallates.

Introduction

Conventional antiviral agents can interfere successfully with viral proteins or functions [1], but exhibit a relatively narrow spectrum of action and often provoke the development of drug resistance in viruses evolving under selective pressures [2,3]. An alternative approach should target antiviral drugs against cellular functions required for virus replication if a transient interference of the normal cell function is well tolerated and results in a retarding of virus replication and spreading [4]. Searching for an antiviral displaying high inhibitory efficiency, low cellular toxicity and low specificity could help to control a wide range of possible virus infections in animals and humans.

Previous studies have shown that gallic acid (3,4,5-trihydroxybenzoic acid) and its alkyl ester derivatives exhibited a potential antitumoural activity on different cultured cells [5–7], inducing apoptotic processes selectively in rapidly growing cells [8] and inhibiting the activity of purified human protein tyrosine kinases [9,10]. Also, the tumouricidal activity of lauryl gallate (LG) towards chemically induced skin tumours has been reported [11]. Some of these compounds, such as propyl gallate (E-310), octyl gallate (E-311) and LG (E-312), have been widely used as antioxidant food additives to prevent the rancidity of different foodstuffs. Regarding the antiviral activity of gallic acid esters, an early study described the inhibition of herpes simplex virus (HSV) by methyl gallate and its inefficiency against several RNA viruses [12]. In addition, two recent publications [13,14] have examined the antiviral effect of several gallic acid esters, with particular attention to octyl gallate, against one DNA (HSV) and three RNA (vesicular stomatitis, influenza and polio) viruses.
We describe in this report the effect of another alkyl ester of gallic acid, LG, on the infectivity of three large DNA viruses (African swine fever virus [ASFV], HSV and vaccinia virus [VV]) and on another three RNA viruses (influenza, Sindbis virus [SV] and porcine transmissible gastroenteritis virus [TGEV]). We also describe its dependence on multiplicity of infection (MOI), the effect of time of addition and the step inhibited by the drug in the virus infection cycle, as determined in the model system of ASFV-infected Vero cell cultures.

Methods

Cells and viruses

Vero (African green monkey kidney), baby hamster kidney (BHK), Madin–Darby canine kidney (MDCK) and swine testis (ST) cells were obtained from the American Type Culture Collection and grown in Dulbecco’s modified Eagle's Medium (DMEM) supplemented with 2 mM l-glutamine, 100 U/ml gentamicin and non-essential amino acids. Swine alveolar macrophages were prepared by bronchoalveolar lavage as previously described [15]. Cells were cultured at 37°C in medium supplemented with either 5% newborn calf serum (Vero), 5% heat-inactivated fetal calf serum (BHK, ST and MDCK) or 10% homologous porcine serum (swine macrophages).

The Vero-adapted ASFV Ba71V strain was propagated and titrated by plaque assay on Vero cells as previously described [16]. Field ASFV isolates (E70, Uganda-vir and Mozam'86) were propagated from frozen stocks on swine macrophages as indicated elsewhere [17,18] and titrated by haemadsorption and plaque assay as previously described [15,16]. Both the KOS strain of HSV, kindly supplied by Dr Fernando Valdivieso (Centro de Biología Molecular Severo Ochoa, Madrid, Spain), and the WR strain of VV were grown and titrated by plaque assay on Vero cells, as indicated [16] for ASFV. Influenza virus (WSN strain), susceptible MDCK cells and the conditions for virus plaque assay were kindly provided by Dr Amelia Nieto (Centro Nacional de Biotecnología, Madrid, Spain). TGEV was provided by Dr Luis Enjuanes (Centro Nacional de Biotecnología).

Virus inhibition assay

To analyse the effect of LG (Sigma–Aldrich, Madrid, Spain) on virus infectivity, the sensitive cells were grown on multiwell plates to approximately 700,000 cells/cm². Stock solutions (40 or 10 mM) of LG were made in ethanol and stored away from light at -20°C and working solutions (1 and 0.1 mM) were freshly prepared in culture medium for each experiment. All the cells were pre-incubated for 1 h with culture medium containing the indicated concentration of LG (0–100 µM) before virus infection. Cultures were then infected with the corresponding virus at the indicated MOI in a reduced volume (approximately 30%) in the presence of the drug for 1–2 h. Virus inoculum was removed and washed twice with saline or culture medium to eliminate non-adsorbed virus and cells were further incubated in drug-containing fresh medium until a massive cytopathic effect was developed in control-infected cultures (in the absence of LG), normally at 24 h post-infection (hpi) in all the DNA viruses tested in this study, and 48–72 h after SV, TGEV or influenza virus infection. Total virus production (or extracellular production for SV and influenza virus) was then titrated in duplicate samples, as described above. Control cultures were infected in parallel in the presence of the same amount of ethanol present in the LG-treated samples.

Cell viability and proliferation assays

To determine the toxic effect of LG treatments in cell culture, we used the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Swine macrophages, Vero, ST, MDCK or BHK cells were seeded on 96-well plates and incubated for 24 h before the addition of LG to produce final concentrations of 0–100 µM in a volume of 0.1 ml per well. Six wells were used for each concentration, and two wells were maintained in parallel with the corresponding medium in the absence of cells (to subtract the background value of drug in the culture medium). After 24 or 48 h of incubation, 90 µl of medium was removed from each well and replaced with 90 µl of fresh medium plus 10 µl of MTT solution (7.5 mg/ml in phosphate-buffered saline [PBS]). Cultures were further incubated at 37°C for 2 h and lysed by adding 100 µl of SDS-containing lysis solution (20% SDS, 0.45 N acetic acid, 0.025 N HCl in N,N-dimethylformamide). After incubation for 15 min with gentle agitation, the absorbance at 550 nm was determined in a microplate reader (Model 680; Bio-Rad Laboratories, Madrid, Spain). Background values were subtracted from the absorbance average value (six wells) obtained for each LG treatment and then compared with that scored in the absence of drug (100% viability).

Cell viability was also determined in LG-treated cultures by trypan blue-exclusion test and results obtained in each cell line were similar to those recorded by MTT assay.

Determination of cellular and viral DNA synthesis

Cultures of Vero cells, either pre-incubated for 1 h with 10 µM LG or not, were mock-infected or infected with ASFV Ba71V strain at an MOI of 5 plaque-forming units (pfu) per cell. After 2 h of adsorption in the presence of the drug, the non-adsorbed virus was washed away from cultures and further incubated at 37°C with LG-containing fresh medium. To study the kinetics of cellular and viral DNA synthesis,
cultures were pulse-labelled for 1 h with methyl-(3H)-thymidine (Amersham GE Healthcare, Barcelona, Spain) at 5 µCi/ml in culture medium. At different times after infection, cells were washed and fractionated with NP40 into nuclear and cytoplasmic fractions as previously described [19]. The acid-insoluble radioactivity was analysed in the nuclear or cytoplasmic fractions to determine the cellular or viral DNA synthesis, respectively.

Western blot analysis
To determine the presence of specific proteins, cultures were collected at different times after virus infection, washed twice with PBS and lysed in TNT buffer (20 mM Tris-HCl, pH 7.5, 0.2 M NaCl, 1% Triton x100) supplemented immediately before use with protease inhibitor cocktail tablets (Roche Farma, Madrid, Spain). Protein concentration was determined by the bicinchoninic acid (BCA) method using the BCA Protein Assay Reagent (Pierce, Cultek, Madrid, Spain). Proteins (30 µg) were subjected to 12% SDS-PAGE, and then electroblotted onto an Immobilon membrane (Amersham). After reacting with primary antibodies specific for early or late ASFV-induced proteins [20], for cellular protein p53 (sc-6243, Santa Cruz Biotechnologies, Heidelberg, Germany) or for cellular active caspase-3 (#559565, BD Pharmingen, San Diego, CA, USA), membranes were exposed to horseradish peroxidase-conjugated secondary antispecies (rabbit or mouse) antibody (Amersham), followed by chemiluminescence (ECL, Amersham) detection by autoradiography.

Results
Evaluation of cellular toxicity and reversibility of LG treatments
The evaluation of cell viability by MTT assay in swine macrophages, Vero, ST, MDCK and BHK cells after incubation with increasing concentrations of LG for 24 or 48 h is shown in Figure 1A. As an estimation of the sensitivity of each cell line to the presence of the drug, we calculated the LG concentration maintaining ≥50% of cell viability (50% cytotoxic concentration [CC50]), which ranged from 20 (BHK, 48 h), 25 (MDCK, 48 h), 30 (ST or swine macrophages, 24 h) to 60 µM (Vero, 24 h). From these results, a working concentration of 10 µM LG was selected to analyse the effect of short-term (24–72 h) treatments of cell cultures. In the case of Vero cells, the effect of the presence of 10 µM LG for several days on cell viability and proliferation is shown in Figure 1B; LG inhibited the growth of Vero cells and maintained its viability for at least 3 days. To test the reversibility of LG treatment, cultures of Vero cells incubated with 10 µM LG for 48 h were released from the drug and further incubated in standard conditions.

Figure 1. Effect of LG on cell viability

(A) Swine macrophages (SM), Vero, swine testis (ST), Madin–Darby canine kidney (MDCK) or baby hamster kidney (BHK) cells grown on 96-well plates were incubated for 24 or 48 h in the presence of increasing concentrations of lauryl gallate (LG), and then analysed for cell viability by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. (B) Vero cells were grown in the presence or absence of 10 µM LG and, at the indicated times, were analysed for cell number and viability by the MTT assay. (C) Vero cells were incubated for 48 h in the presence of 10 µM LG, then released from the drug and further incubated for 5 additional days in the absence of LG. Control cultures incubated in the absence of LG were maintained in parallel.
The recovered cells exhibited the same growth kinetics (Figure 1C) and ASFV sensitivity (data not shown) than that of untreated control Vero cells.

Effect of LG on DNA and RNA virus productivity
We first analysed the effect of different concentrations of LG on the virus productivity of three DNA viruses (ASFV, HSV and VV) infecting Vero cells (Figure 2A). An almost undistinguished dose-dependent inhibition curve was obtained in all of the viruses tested, suggesting that the same process was affected by the drug in all of the virus infections. The residual presence of solvent (ethanol) did not affect virus production, even at a concentration corresponding to 100 μM LG, whereas antiviral activity was titrated between 2–30 μM LG, a concentration that maintained the viability of Vero cells >90%.

To determine if the antiviral drug might be used for in vivo ASFV infections, we studied the effect of LG on
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virus productivity of several ASFV field isolates grown in swine alveolar macrophages. Although the presence of the drug was slightly more toxic in these cultures (CC50 30 µM) than in Vero cells (CC50 60 µM), antiviral activity was still observed between 2–10 µM LG, a range already outside cell toxicity (Figure 2B). The effective concentration of LG to reduce 50% (EC50) or 90% (EC90) of virus productivity was estimated to be 2–3 µM and 4 µM, respectively, for all DNA viruses tested (Figures 2A & 2B).

The inhibition of the RNA-containing SV by the presence of the drug was then analysed after incubation with increasing concentrations of LG in BHK cells infected at two different MOIs (0.5 or 2 pfu per cell). As shown in Figure 2C, infective virus titres decreased approximately 2 log units after 48 h in the presence of 10 µM LG (CC50 for BHK cells was 20 µM LG in the assay conditions). A more dramatic effect was observed in the WSN strain of influenza virus, produced on MDCK cell cultures. Figure 2D shows that the degree of virus inhibition was extended in this case to >5 log units, in cells infected at MOI 0.001 pfu per cell between 3–10 µM LG (non-toxic concentrations for MDCK cells with a CC50 of 25 µM). However, the inhibitory EC of LG (Figures 2C & 2D) estimated for both RNA viruses was similar (1–3 µM for EC50 and 4–5 µM for EC90) to that obtained in the infection by DNA viruses.

Effect of MOI and time of addition on the inhibiting activity of LG

As shown above, the average inhibition of DNA virus productivity by LG treatment (10 µM) in our standard assay was 3 log units. Because the standard test in DNA viruses was carried out at a relatively high MOI (approximately 2 pfu per cell), we thought it would be of interest to know the effect of LG at a lower MOI, considering that the first stages in virus disease should correspond to infections with a very low ratio of pfu per susceptible cell. To achieve this, we have used the model system of ASFV-infected Vero cells, in which we found that the efficiency of LG-mediated inhibition of virus productivity was higher when lower MOIs were used. Decreases of ≥3, 4 and 5 log units were obtained when the assay was performed at a MOI of 2, 0.2 or <0.5 pfu per cell, respectively (Figure 3A).

In the case of RNA viruses, the assay of inhibition was made at different MOIs depending on the particular virus system, as indicated in the previous section (and in the legend of Figure 2). Dose-dependent inhibition of virus production was detected (Figures 2C & 2D) in the presence of LG in cell cultures infected by SV (MOI 0.5–2 pfu per cell) and influenza virus (MOI 0.001 pfu per cell). We also analysed another RNA virus (TGEV), in which preliminary experiments...
indicated that the virus production on ST cells was not largely affected by LG treatments in the standard virus inhibition assay (data not shown). However, when the experiment was done at different MOIs (Figure 3B) it was observed that the highest inhibition of virus production (90–95%) was obtained in ST cells infected at a very low MOIs (10⁻⁴ and 10⁻⁵ pfu per cell) in the presence of 5 or 15 μM LG, whereas MOIs≥10⁻³ pfu per cell resulted in only weak reductions (to about one half) of virus titres. The viability of ST cells (CC₅₀ of 30 μM LG) remained close to 100% at the LG concentrations used in the experiment (Figure 1A).

To determine the step of the virus cycle affected by the presence of LG, we performed an experiment in which the drug (10 μM) was added to cultures of ASFV-infected Vero cells at different times before or after infection (-5–24 h), titrating the total virus produced at 24 hpi. As shown in Figure 3C, no virus production was detected when LG was added between -5–4 hpi (and maintained throughout the rest of the cycle), whereas the drug was completely ineffective when added 12–24 hpi, indicating that the step affected by LG occurs between 5–8 hpi in the ASFV infectious cycle.

Effect of LG on a one-step ASFV cycle

We selected the model system ASFV-infected Vero cells to perform a more detailed study of the effect of LG on the virus infectious cycle. The analysis of the growth curve in a one-step viral infection (Figure 4A) showed that extracellular infective virus particles were not produced in the presence of the drug, suggesting that virus dissemination might have been blocked by LG. However, the cells infected in the presence of the drug developed an extensive cytopathic effect, and eventually lysed at later times of infection.

To determine the precise step inhibited by LG in the infectious cycle, we analysed the presence of ASFV-specific early and late proteins induced in Vero cells infected by ASFV in the presence or absence of the drug. As shown in Figure 4B, both early (vp32) and late virus-specific proteins were detected, as expected, at different times of infection in control ASFV-infected cell cultures, whereas the presence of LG resulted in the inhibition of the late virus-induced proteins. Moreover, the induction of early proteins specific for ASFV should account for the cytopathic effect observed in LG-treated ASFV-infected Vero cell cultures, as occurred, for example, when the virus infection was performed in the presence of cytosine arabinoside, which blocked the late stages of the ASFV infection [21].

We next examined whether cellular and viral DNA synthesis was affected by the drug treatment. The results of pulse-labelling experiments in Vero cells infected by ASFV indicated that both nuclear and cytoplasmic DNA synthesis was drastically inhibited by LG (Figure 4C).

It is important to consider that viral DNA synthesis occurred mainly in the cytoplasm of the infected cell, and began after 4–5 hpi: for this reason, it could not be inhibited (because it is not induced) at very early times of infection, as shown in Figure 4C. The inhibition of cellular DNA synthesis by LG in mock-infected cultures could explain the observed cell growth arrest (Figure 1B), representing a very efficient, but reversible (Figure 1C), blockade of DNA replication.

Effect of LG on the induction of cellular proteins p53 and caspase-3

We previously described the induction of the tumour suppressor protein p53 [22] and the pro-apoptotic effector caspase-3 [21] in ASFV-infected Vero cells. To determine whether the LG treatment might affect the activation of these cellular factors, which are crucial in the control of cell cycle and apoptosis, Vero cells were infected with ASFV in the presence or absence of the drug and tested by western blot for the expression of p53 and caspase-3 at 4 and 16 hpi. As shown in Figure 5A, the presence of LG did not affect the increase of p53 induced in Vero cells by ASFV infection. Interestingly, Vero cells incubated in the presence of LG did not result in caspase-3 activation, indicating that the apoptotic process was not induced by the drug in non-infected cultures. Moreover, LG also inhibited the activation of caspase-3 induced by ASFV in Vero cells at late times of infection (16 hpi; Figure 5).

Discussion

We describe in this paper that short-term treatments with a therapeutical dose (10 μM) of LG resulted in the inhibition of Vero cell proliferation without affecting the cell viability, a transient situation that could be recovered to normal parameters of cytoproliferation and virus sensitivity by simply releasing the cell culture from the drug.

The main observation in this report was the efficient inhibition of virus production observed in several cell lines incubated in the presence of LG. A wide spectrum of viruses, of both veterinary and clinical importance, were inhibited by alkyl gallates, including members from many different families like Poxviridae, Asfarviridae, Herpesviridae, Orthomixoviridae, Togaviridae and Coronaviridae, reported here, and Rhabdoviridae, Picornaviridae, Herpesviridae and Orthomixoviridae, which have been reported in previous articles [12–14]. In the case of DNA viruses we have shown a very similar dose-dependent inhibition of the yield of infective ASFV, VV or HSV virions in Vero cell cultures infected in the presence of LG, extending the inhibitory activity to several field isolates grown in swine macrophages in the case of ASFV. The similarity of the inhibition curves...
obtained for all three DNA viruses tested on Vero cells, and the efficient inhibition of DNA- and RNA-containing viruses belonging to such a broad spectrum of virus families, suggests that a cellular process (more so than a specific virus component) was the target in LG treatments, although the possibility that a similar factor or process common to all the viruses might be blocked by the drug cannot be completely excluded.

Because HSV has been studied in several reports of antiviral activity of alkyl gallates, we have calculated the antitherpetic EC\textsubscript{50}. The data obtained were 674–2,354 \mu M (depending of the virus strain [12]) or 3.5 \mu M [13] for octyl gallate and 2.0 \mu M for LG in this report. Besides being the most effective agent against HSV, the alkyl ester LG was also efficient against other important clinical pathogens, such as influenza virus, and against viral agents involved in veterinary diseases, such as ASFV and TGEV in swine.

Furthermore, we have paid attention to the effect of putative antiviral drugs on host cell viability in order to

Figure 4. Effect of LG on a one-step ASFV cycle

(A) Growth curve. Vero cells were either pre-incubated for 1 h with 10 \mu M lauryl gallate (LG), or not, and infected with the African swine fever virus (ASFV) Ba71V strain at a multiplicity of infection (MOI) of 0.5 plaque-forming units (pfu) per cell. Non-adsorbed virus was washed away and the cultures maintained in the presence or absence of LG to complete the virus cycle. At the indicated times after infection the extracellular virus production was titrated by plaque assay on Vero monolayers.

(B) Protein expression. Cell extracts obtained at different hours post-infection (hpi) from samples infected as indicated in (A) were subjected to western blot analysis to detect early (vp32) or late ASFV-induced proteins in the presence or absence of LG. The presence of early or late virus-induced proteins is indicated by arrows.

(C) DNA synthesis. To determine the specific cellular and viral DNA synthesis, cultures of Vero cells were mock-infected or infected with the ASFV Ba71V strain at an MOI of 5 pfu per cell, in the presence or absence of LG, as indicated in (A). Cultures were pulse-labelled for 1 h at different times after infection with methyl-\textsuperscript{3}H-thymidine, and separated into nuclear and cytoplasmic fractions. DNA synthesis is represented by the ratio between the trichloroacetic acid (TCA)-insoluble radioactivity obtained in the LG-treated mock (m+LG) or infected (V+LG) samples versus the corresponding non-treated cultures (m or V), using the value obtained at 0 hpi in each series as a reference for 100%. Cellular DNA synthesis corresponds to the data in nucleus from non-infected cultures (m+LG/m), whereas viral DNA synthesis is best represented in the cytoplasm of infected samples (V+LG/V).
exclude side effects of cellular toxicity and to the return
to normal status after releasing the treatment in order
to guarantee that cells are only transiently affected by
the antiviral treatments. This is a particularly impor-
tant issue when the antiviral product is directed against
细胞 components or functions. Therefore, we have
included in this study the dose-response curve of the
cytotoxic effect of LG on each cell monolayer used
to determine the specific range of LG concentrations
resulting in antiviral activity without cytotoxicity. It is
important to note that this is an absolute requirement
when assessing the ability of any drug to be considered
for antiviral therapy that, indeed, might behave differ-
ently in each particular cell and virus system. This
information was not considered in previous reports
on the antiviral effect of another alkyl (octyl) gallate
against DNA and RNA viruses [13,14], and might
be crucial in the interpretation of results on assays of
direct virucidal activity of octyl gallate, as they were
performed at 100–400 µM in the presence of concen-
trations of solvent dimethyl sulfoxide that were possi-
bly toxic (dilution 1/10). Information on the cytotoxic
effect of LG is also important for understanding the
inhibition to approximately 20% in polio virus yield in
Hep-2 cells, obtained in a concentration of octyl gallate
(40 µM), that resulted, in the case of LG on BHK
or MDCK cells, in a massive cell death (>50% cytotox-
icity in 24 h incubation, as estimated by MTT assay).
Indeed, it was pointed out by the authors [13] that the
inhibitory effect could be, at least in part, the result of
non-specific degeneration of the infected cells.

In order to consider future applications of LG in
antiviral therapy, it was important to determine the
degree of inhibition of infective virus yield and the
stage of the virus cycle blocked by the drug. These
particular tests were performed in the model system of
ASFV-infected Vero cells. In this regard, the absence
of extracellular infective virus particles in a one-step
ASFV infection on Vero cells suggests that no virus
dissemination should be expected when the cell is
infected in the presence of LG. Considering the low
MOI associated with the in vivo infection in field con-
ditions, this represents minimal damage in the whole
organism, as no infectious virus would be produced
after the first round of infection.

The inhibition of infective virus production was in
agreement with the absence of induction of ASFV-
specific late proteins in the presence of LG, proba-
bly as a direct consequence of the drastic inhibition of
the synthesis of viral DNA. Interestingly, the DNA
synthesis in the cell nucleus was also blocked by LG,
indicating that DNA replication was completely pre-
vented by the drug and supporting the antitumoural
activity associated with the drug [5]. From our results
it can be concluded that ASFV infection proceeded
normally in Vero cells, in the presence of LG, from
the attachment of virus particles, internalization and
uncoating of virus cores, to complete the transcription
of early virus proteins throughout the first 5–8 hpi. By
this time, an LG-sensitive process would be required
to continue with the virus infection that, in the case
of ASFV, would lead to the viral DNA replication at
10–12 hpi. In line with this interpretation, the addition
of the drug was less effective at 8 hpi and inefficient
at 12 hpi, consistent with the reported data from octyl
gallate-treated Hep-2 cells infected with HSV that
were fully productive if the drug was added at 8 hpi
[13]. Moreover, Yamasaki et al. [14] described the
inhibition by octyl gallate of a step in the middle stage
of the multiplication of influenza virus. The inhibition
of a similar step by two alkyl gallates in the infection
of different cells by several DNA and RNA viruses is
almost incompatible with the hypothesis of a virus-
specific component as target for the antiviral activity;
most probably, a cellular factor or function that
is affected by the drug treatment is required in all of
the viral infections susceptible to that type of antiviral
drug. Accordingly, it could be expected that different
viruses will display varied sensitivities to the drug, in
correspondence with the specific requirement of each
virus on the affected cell function. Indeed, differences
in the efficiency of alkyl gallates to inhibit two RNA
viruses, such as vesicular stomatitis virus and polio-
virus or influenza and TGEV, have been described by
Uozaki et al. [13] and also in this report.

Regarding the mechanism by which the alkyl gallates
might inhibit the virus productivity, previous reports
described the induction of apoptosis in tumoural

Figure 5. Effect of LG on the induction of p53 and caspase-3

Vero cells were mock-infected or infected with the African swine fever virus (ASFV) Ba71V strain at a multiplicity of infection (MOI) of 5 plaque-forming units (pfu) per cell, either in the presence or absence of 10 µM lauryl gallate (LG). Cell extracts were obtained at 4 and 16 h post-infection (hpi) and subjected to western blot analysis to detect the cellular proteins p53 and caspase-3 (casp-3). A sample as positive control (C+) for the induction of p53 and casp-3 was run in parallel.
cell lines by derivatives of gallic acid [5,8] and the concomitant inhibition of the protein tyrosine phosphorylation detected in the presence of pavanadate. In the case of Vero cells, we have not been able to detect the activation of caspase-3 after incubation of cell cultures with non-toxic concentrations of LG, indicating that the apoptotic process was not triggered by the drug. This interpretation was also supported by the reversibility of the effects of LG on Vero cells (growth arrest and inhibition of DNA synthesis) when the cultures were released from the drug. As expected, incubation with LG, which completely inhibited ASFV infection in our study, resulted in the blocking of the caspase-3 activation induced after 13 hpi in Vero cells [23]. Assuming that the target for LG is a cellular factor, we believe that the inhibition of protein kinases is a possible effect of LG, as the blockade of protein phosphorylation could produce many of the reported results; activation of proteins by kinases is one of the most important regulatory events in the eukaryotic cell, crucial, for example, in the triggering of the apoptotic cascade. Determining the precise kinase(s) affected by antiviral drugs will be the primary objective of our investigations in the future.

Acknowledgements

This work was supported by grants from Ministerio de Educación y Ciencia, Spain (BFU2004-00298/BMC), Laboratorios del Dr Esteve, Barcelona, Spain and by institutional grants from Fundación Ramón Areces, Madrid, Spain and Banco Central Hispano, Madrid, Spain. CH was a fellow from Fundación Ramón Areces. AGG was funded by Centro de Investigación en Sanidad Animal (CISA), Valdeolmos, Spain.

Disclosure statement

The authors declare no competing interests.

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