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# Three-dimensional analysis of combination effect of ellagitannins and acyclovir on herpes simplex virus types 1 and 2

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ABSTRACT

The effects of combinations of three nonahydroxyterphenoyl-bearing *C*-glucosidic ellagitannins (castalagin, vescalagin and grandinin) with acyclovir (ACV) on the replication of type-1 and type-2 *herpes simplex* viruses in MDBK cells were tested by the focus-forming units reduction test. Ellagitannins included in these combinations possess a high individual antiviral activity: selectivity index of castalagin and vescalagin versus HSV-1 was similar to that of ACV, and relatively lower against HSV-2. The three-dimensional analytical approach of Prichard and Shipman was used to evaluate the impact of drug–drug interactions. The combination effects of ellagitannins with acyclovir were markedly synergistic.



## 1. Introduction

*Herpes simplex* viruses of type 1 (HSV-1) and type 2 (HSV-2) are important pathogens for humans, especially in the case of immunocompromised patients. Moreover, HSV-2 infection has been reported to increase the risk of human immunodeficiency virus (HIV) transmission. HSV-1 and HSV-2 are the primary agents of recurrent facial and genital herpetic lesions (Roizman and Knipe, 2001), HSV-2 infection being classified as a sexually transmitted disease. For immunocompromised patients and neonates, this will often results in painful and disabilitating lesions, and in some cases death (Whitley, 1995; Andersen et al., 2003).

Acyclovir (ACV) was the first effective virus-specific antiherpes drug made available. Afterwards, other nucleoside analogues have been developed in the aim of exhibiting better bioavailability than that of ACV (Efstathiou et al., 1999; Crumpacker, 2001; De Clerq, 2001). These analogues have been used with some success in the treatment of mucocutaneous and ocular HSV infections (Cremonesi et al., 1994). Although there is at present no effective vaccine or drug capable of fully eradicating established HSV infections, these antiviral drugs are able to shorten the course and decrease severity of symptomatic episodes in both normal and immunocompromised patients (Malkin, 2002). As far as the clinical use of antiherpetic nucleoside analogues as ACV is concerned, their efficacy is often compromised by the appearance of drug-resistant HSV mutants in immunosuppressed patients, such as organ transplant recipients and patients with AIDS (Coen, 1994; Bacon et al., 2003). Therefore, it is of crucial importance to search for novel compounds with alternative mechanisms of antiviral action. At the same time, treatments based on the combination of different antiviral agents are considered as promising approaches to limit the manifestation of drug resistance (Freestone, 1985; Mucsi et al., 2001; Gong et al., 2004) and to increase the antiviral effect selectivity by decreasing the active drug concentration (Allen et al., 1982; Talarico et al., 2006).

Ellagitannins constitute a class of plant polyphenols composed of a central sugar core, typically β-D-glucose, which is acylated by galloyl units that are further connected through C–C biaryl and C–O diaryl ether bonds (Quideau and Feldman, 1996; Quideau, 2009; Quideau et al., 2010). Numerous members of this class of so-called hydrolyzable tannins have been identified as active principles in plant extracts used in traditional oriental medicines (Okuda et al., 1981, 1989; Haslam et al., 1989; Okuda, 1999). A significant number of these ellagitannins expressed antitumoral activities (Miyamoto et al., 1993; Yang et al., 1999; Ito et al., 2007), as well as antiviral activities, in particular against HIV infection (Nonaka et al., 1990; Nakashima et al., 1992; Martino et al., 2004; Notka et al., 2004). Moreover, some ellagitannins manifested inhibitory effects on replication of HSV-1 and/or HSV-2, as well as Epstein-Barr virus (EBV) (Takechi et al., 1985; Fukuchi et al., 1989; Corthout

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et al., 1991; Kurokawa et al., 1998, 2001; Liu et al., 1999; Cheng et al., 2002; Ito et al., 2007). In a previous work, Quideau et al. (2004) reported on the anti-herpesvirus activity of members of a particular subclass of *C*-glucosidic ellagitannins, composed of an open-chain glucose acylated by a galloyl-derived nonahydroxyterphenoyl unit (NHTP, also known as flavogalloyl group). Some of these substances (e.g. castalagin, vescalagin and grandinin) displayed a marked inhibitory effect on the replication of HSV-1 and HSV-2, including ACV-resistant strains. It was then of special interest to investigate the combination effects of these compounds with ACV against HSV-1 and HSV-2 strains, which is the topic of this communication.

## 2. Materials and methods

### 2.1. Cells

Monolayer cultures of Madin-Darbey bovine kidney (MDBK) cells (National Bank for Industrial Microorganisms and Cell Cultures, Sofia) were grown in DMEM medium containing 10% fetal bovine serum Gibco BRL, USA, supplemented with 10 mM HEPES buffer (Merck, Germany) and antibiotics (penicillin 100 IU/ml, streptomycin 100 μg/ml) in $CO_2$ incubator (HERA cell 150, Heraeus, Germany) at 37 °C/5% $CO_2$.

### 2.2. Viruses

Herpes simplex virus type 1, Victoria strain (HSV-1) and herpes simplex virus type 2, strain Bja (HSV-2) was received from Prof. S. Dundarov, National Center of Infectious and Parasitic Diseases, Sofia. Viruses were replicated in monolayer MDBK cells in a maintenance solution DMEM Gibco BRL, Paisley, Scotland, UK, plus 0.5% fetal bovine serum Gibco BRL, Scotland, UK. Infectious titer of stock viruses was $10^{6.5}$ and $10^{6.75}$ $CCID_{50}$ (50% cell culture infectious doses) for HSV-1 and HSV-2 strains, respectively.

### 2.3. Compounds tested

Nonahydroxyterphenoyl-containing *C*-glucosidic ellagitannins: castalagin, vescalagin and the lyxose-containing grandinin (Fig. 1), extracted from powdered pedunculate oak (*i.e.*, *Quercus robur*) heartwood and purified as previously described (Quideau et al., 2004), were first dissolved in distilled water to a concentration of 0.01 M and then diluted in DMEM to the required concentration. Acyclovir [9-(2-hydroxyethoxymethyl)-guanine] (ACV) was also dissolved in DMEM to the required concentration.

### 2.4. Cytotoxicity assays

The *in vitro* cytotoxicity on MDBK cell culture of ellagitannins and ACV was examined on both confluent monolayer and growing cells.

#### 2.4.1. Cytotoxicity assays in resting cells

Confluent monolayer cell cultures in a 96-well plate were treated with culture medium containing either no antiviral agent or increased concentrations of ellagitannins and ACV. The viability of the cells after drug treatment was measured using three assays resulting in $CC_{50}$ values evaluation: (a) neutral red uptake assay based on the initial protocol described by Borenfreund and Puerner (1984) using ELISA reader at $OD_{540\,nm}$ (measurement after incubation of 48 and 72 h); (b) lactate dehydrogenase (LDH) leakage assay (done at the 24th and 48th h after the treatment onset) used to determine the activity of LDH released in the medium using a commercially available kit from Sigma–Aldrich® (TOX7). The amount of LDH activity is an indicator of relative cell viability as well as a function of membrane integrity. This assay is based on the reduction of NAD by LDH to NADH, which is involved in the conversion of a tetrazolium dye to a red colored compound (formazan), the amount of which being measured spectrophotometrically at $OD_{490\,nm}$ (after incubation of 24 and 48 h); (c) MTT assay based on the protocol described for the first time by Mossmann (1983) and optimized for the MDBK cell line. At the end of the incubation time (on the 24th, 48th and 72nd h of treatment with the substance tested), a 1 mg/ml solution of MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] was added (100 μl per well) to the culture maintenance solution and cells were incubated for 4 h. The yellow water soluble MTT dye is reduced by live cells to a water insoluble purple formazan. The amount of formazan, solubilized in isopropanol, is measured spectrophotometrically at $OD_{540\,nm}$. Each of the tests described above was done in triplicate to quadruplicate, with four cell culture wells per test sample.

#### 2.4.2. Cytotoxicity assays in growing cells

The effect of substances tested on the growth curve of MDBK cells was examined using two techniques with evaluation of the 50% cell growth inhibitory concentration ($CGIC_{50}$) value: (a) by measurement of the cell count on the 24th, 48th and 72nd h after the seeding of $10^4$ cells per well in 24-well plates (1 ml of a mixture 1:1 in the growing medium of $2 \times 10^4$ cells + substance tested at double concentration); (b) by neutral red uptake assay (as described in Section 2.4.1) in 96-well plates (200 μl 1:1 $2 \times 10^4$

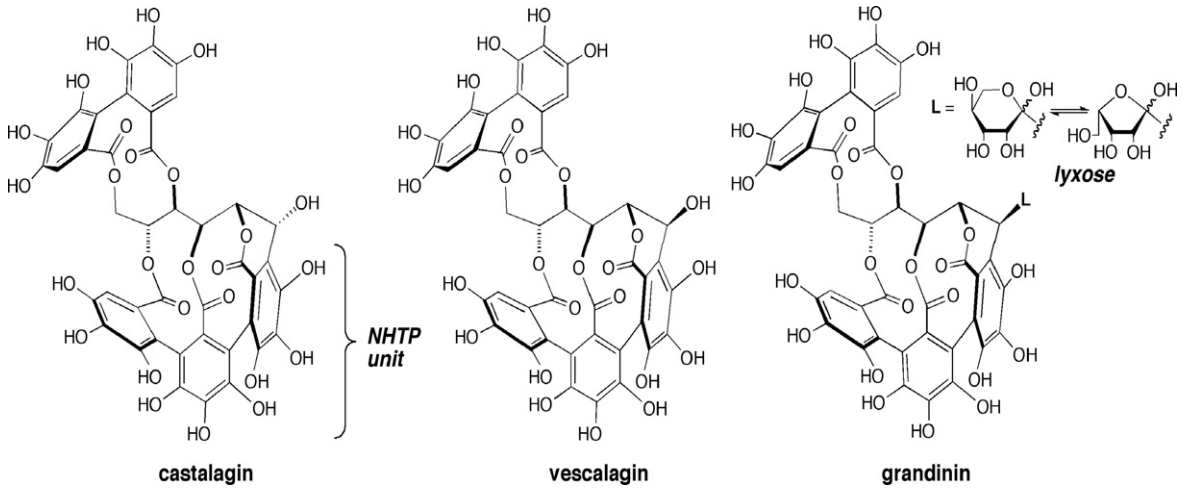


**Fig. 1.** Molecular structure of the ellagitannins.

cells + substance tested at double concentration) done at the 24th, 48th and 72nd h after the cell seeding. Each of the two tests was done in triplicate with four cell culture wells per test sample.

### 2.5. Antiviral activity assay

Two methodical approaches were used for assessment of the ellagitannins' anti-herpesvirus activity. According to the focus forming units (FFU, *i.e.*, microscopically registered microplaques) reduction assay, monolayer MDBK cells ($4 \times 10^4$ per well) grown in 96-well plates were inoculated with 100 $CCID_{50}$ per well (MOI = 0.0025). After 60 min of incubation at 37 °C, infected cells were treated with the test compounds applied either alone or in pair combinations with ACV. After 48 h of incubation at 37 °C, the FFU number in the monolayer was counted under light microscope and the inhibition effect in percentage was evaluated by comparison with the controls not treated with any substance.

Cytopathic effect (CPE) inhibition test used confluent cell monolayer in 96-well plates infected with 100 $CCID_{50}$ in 0.1 ml (MOI = 0.0025). After 1 h of virus adsorption, compounds were added in various concentrations and cells were incubated for 48 h at 37 °C. Inhibition of cytopathic effect was determined using a neutral red uptake assay and an ELISA reader at $OD_{540\,nm}$. The $IC_{50}$ concentration of the ellagitannins and ACV was identified as that concentration that inhibited development of CPE by 50%.

### 2.6. Combination effects analysis

Antiviral combination effects due to drug–drug interaction were examined by relying on the three-dimensional model system developed by Prichard and Shipman (Prichard and Shipman, 1990; Nikolaeva and Galabov, 1999) by using the computer program MacSynergy™ II (Prichard et al., 1992). This program uses confidence intervals to statistically evaluate the drug interactions. The additivity assumption equations for both single and different site inhibitor(s) were used. The difference between the observed combination effects and those expected if effects were simply additive was calculated. The resulting surface would appear as a horizontal plane at 0% inhibition. A peak above the zero surface represents greater than additive antiviral activity (*i.e.*, synergy), whereas any depression below the zero surface indicates antagonism (Prichard and Shipman, 1990). To depict the statistically significant synergy and/or antagonism at 95%, all points that are not significant at this confidence interval are arbitrarily set to zero (*i.e.*, in the statistical plots, any point that appears above or below the plane at 0% lies outside the confidence envelope). The programme also calculates the volume of synergy and/or antagonism in $\mu M^2\%$: (i) values of synergy or antagonism under 25 $\mu M^2\%$ at 95% confidence should be regarded as insignificant; (ii) values between 25 $\mu M^2\%$ and 50 $\mu M^2\%$ should be considered as indicative of a minor but significant amount of synergy; (iii) values between 50 $\mu M^2\%$ and 100 $\mu M^2\%$ indicate moderate synergy or antagonism (this interaction may be important *in vivo*); (iv) values over 100 $\mu M^2\%$ indicate strong synergy (they are probably important *in vivo*) (Prichard et al., 1992). Combination antiviral effects were evaluated through the FFU reduction method. The character of the combination cytotoxicity in MDBK cell monolayers was determined following the 3D model system as described above by applying the neutral red uptake assay. The experiments both in antiviral and cytotoxicity testing (Sections 2.4–2.6) are performed four times in quadruplicate.

### 2.7. Statistical analysis

Data on compounds cytotoxicity and antiviral effects were analysed statistically. The values of $CC_{50}$, $CGIC_{50}$ and $IC_{50}$ were presented as means ± SD. The differences' significance between the cytotoxicity values of ellagitannins and ACV as well as between the effects of compounds on HSV-1 and HSV-2 was done through the Student's *t*-test. *p*-values of <0.05 were regarded significant.

## 3. Results

The cytotoxicity of the three ellagitannins for MDBK cells was studied both in stationary and growing cell cultures, ACV been tested in parallel. The cytotoxicity for the cell cultures in stationary phase was measured through three methods: neutral red uptake, LDH leakage and MTT assays. As seen in Table 1A, ellagitannins are more cytotoxic than ACV, $CC_{50}$ values of these compounds been markedly inferior than that of ACV ($p < 0.0001$; Student's *t*-test). The lowest $CC_{50}$ values of ellagitannins were recorded by MTT test, the highest by LDH leakage test, data obtained by neutral red test occupied the intermediary position. ACV manifested lowest $CC_{50}$ values in the neutral red test and the highest in the LDH leakage test.

**Table 1**
Cytotoxicity of ellagitannins and ACV for MDBK cells viability.

(A) In monolayer cell culture

| Compound | $CC_{50}^{NR}$ ± SD (μM)[a] | | $CC_{50}^{LDH}$ ± SD (μM)[b] | | $CC_{50}^{MTT}$ ± SD (μM)[c] | | |
|---|---|---|---|---|---|---|---|
| | 48 h | 72 h | 24 h | 48 h | 24 h | 48 h | 72 h |
| Castalagin | 53.9 ± 4.2 | 41.9 ± 3.9 | 79.5 ± 5.4 | 63.1 ± 4.9 | 45.2 ± 5.9 | 39.5 ± 2.6 | 35.1 ± 3.1 |
| Vescalagin | 68.2 ± 3.9 | 54.0 ± 4.4 | 81.2 ± 3.5 | 85.5 ± 1.6 | 44.2 ± 6.5 | 27.0 ± 1.6 | 26.2 ± 2.1 |
| Grandinin | 35.5 ± 5.2 | 29.9 ± 2.9 | 100.0 ± 6.3 | 96.4 ± 5.7 | 50.4 ± 2.5 | 36.6 ± 8.2 | 32.8 ± 8.2 |
| ACV | 1296.0 ± 49.4 | 506.0 ± 17.0 | >3000 | >3000 | >3000 | 1963.0 ± 175.7 | 666.5 ± 75.6 |

(B) In growing cells

| Compound | $CGIC_{50}^{CCT}$ ± SD (μM)[d] | | | $CGIC_{50}^{NR}$ ± SD (μM)[e] | | |
|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| Castalagin | 21.9 ± 2.2 | 10.6 ± 2.1 | 3.9 ± 0.7 | 17.8 ± 1.1 | 13.4 ± 1.3 | 5.2 ± 1.3 |
| Vescalagin | 20.2 ± 2.3 | 19.3 ± 0.5 | 14.9 ± 2.0 | 20.0 ± 1.1 | 19.0 ± 0.7 | 17.2 ± 1.6 |
| Grandinin | 15.3 ± 1.5 | 5.4 ± 0.7 | 3.9 ± 0.8 | 17.3 ± 1.6 | 8.8 ± 1.3 | 4.5 ± 1.1 |
| ACV | 618.0 ± 28.1 | 380.0 ± 17.2 | 213.0 ± 11.4 | 694.5 ± 22.9 | 413.0 ± 11.8 | 221.0 ± 13.3 |

[a] Tested by neutral red uptake assay.
[b] Tested by lactate dehydrogenase leakage assay.
[c] Tested by MTT assay.
[d] Cell growth registered by cell counting.
[e] Cell growth registered by neutral red uptake assay.

**Table 2**
Individual effect of ellagitannins and ACV on the replication of HSV-1 (Victoria), HSV-2 (Bja) in MDBK cells.

| Compound | HSV-1 | | HSV-2 | |
|---|---|---|---|---|
| | $IC_{50} \pm SD$ (μM) | | $IC_{50} \pm SD$ (μM) | |
| | FFU reduction test | CPE inhibition test | FFU reduction test | CPE inhibition test |
| Castalagin | $0.012 \pm 0.003^{***,\hat{}\hat{}\hat{}}$ | $0.058 \pm 0.012^{***,\hat{}\hat{}}$ | $0.6 \pm 0.04^{**}$ | $0.88 \pm 0.07^{*}$ |
| Vescalagin | $0.075 \pm 0.007^{***,\hat{}\hat{}}$ | $0.18 \pm 0.03^{***,\hat{}\hat{}\hat{}}$ | $0.55 \pm 0.06^{**}$ | $0.94 \pm 0.05^{**}$ |
| Grandinin | $0.4 \pm 0.06^{**,\hat{}}$ | $0.88 \pm 0.04^{***,\hat{}}$ | $0.5 \pm 0.06^{**}$ | $0.78 \pm 0.05^{**}$ |
| ACV | $1.33 \pm 0.09^{\hat{}}$ | $1.47 \pm 0.06^{\hat{}}$ | $1.6 \pm 0.09$ | $1.74 \pm 0.12$ |

$^{*}$ $p < 0.05$ when comparing each of the ellagitannins tested to ACV; Student's *t*-test.
$^{**}$ $p < 0.001$ when comparing each of the ellagitannins tested to ACV; Student's *t*-test.
$^{***}$ $p < 0.0001$ when comparing each of the ellagitannins tested to ACV; Student's *t*-test.
$^{\hat{}}$ $p \leq 0.05$ comparison between the compounds' effect against HSV-1 and against HSV-2; Student's *t*-test.
$^{\hat{}\hat{}}$ $p < 0.001$ comparison between the compounds' effect against HSV-1 and against HSV-2; Student's *t*-test.
$^{\hat{}\hat{}\hat{}}$ $p < 0.0001$ comparison between the compounds' effect against HSV-1 and against HSV-2; Student's *t*-test.

Effects of ellagitannins and ACV on the growth curve of MDBK cells are shown in Table 1B. Results obtained manifest a markedly inferior $CGIC_{50}$ values for the four substances as compared with their $CC_{50}$ values. Close $CGIC_{50}$ values were established by the two evaluation approaches, direct cell counting under light microscope and cell viability measurement in the neutral red test. The markedly higher cytotoxicity of ellagitannins as compared with ACV ($p < 0.001$; Student's *t*-test) was confirmed in growing cells, as well.

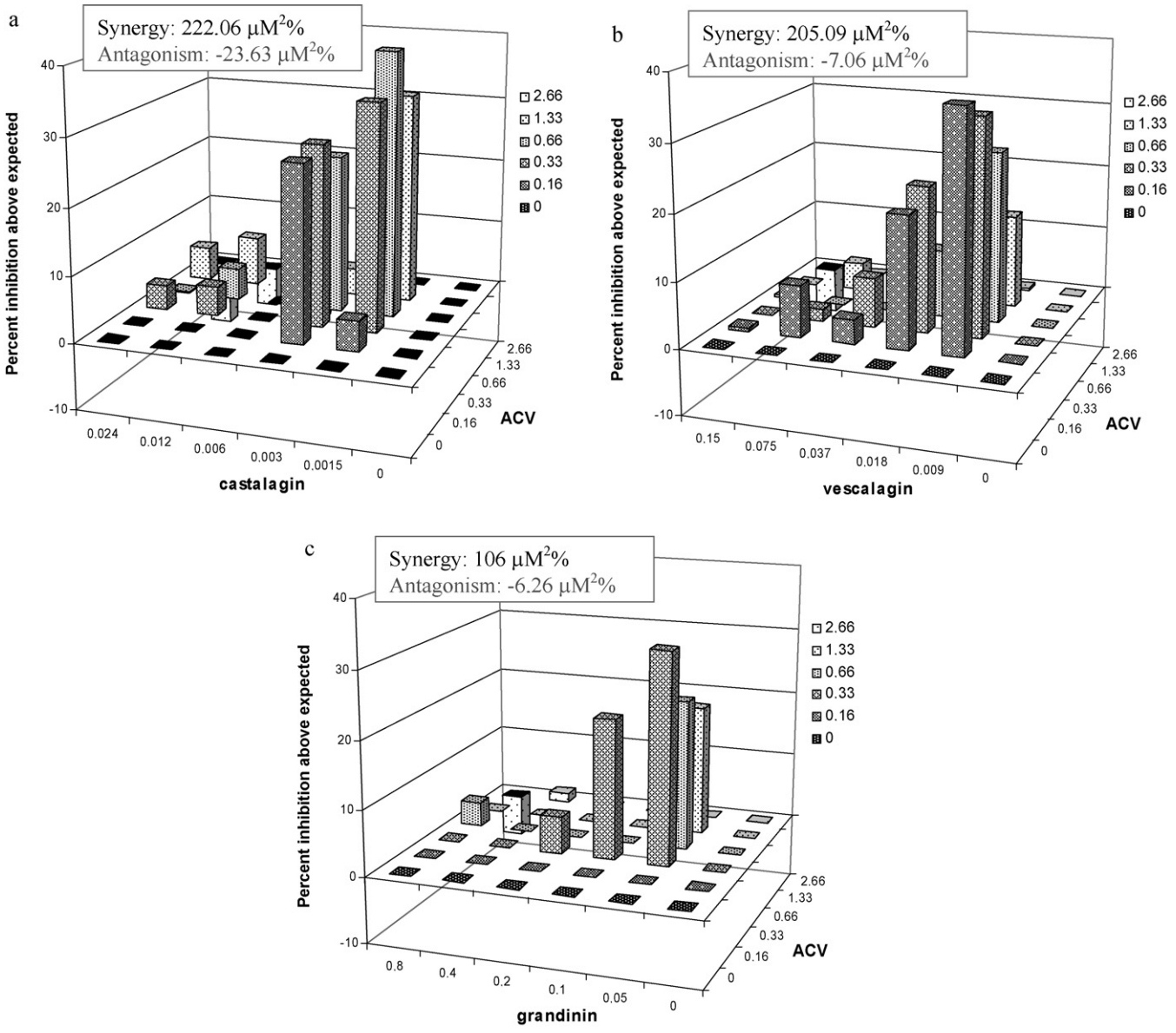


**Fig. 2.** Effect of combinations of ellagitannins and ACV on HSV-1 (Victoria strain) replication: (a) castalagin and ACV; (b) vescalagin and ACV; (c) grandinin and ACV.

The ellagitannins tested, vescalagin, castalagin and grandinin (Fig. 1), demonstrated pronounced inhibitory effects on the replication of both HSV-1 (Victoria strain) and HSV-2 (Bja strain), as measured by the two methods applied, FFU reduction assay and CPE inhibition test (Tables 2 and 3). Selectivity evaluation (SI) of the compounds was illustrated in more details including $CC_{50}$ values obtained in resting cell culture (Table 3A) and $CGIC_{50}$ in growing cells (Table 3B). As shown in Tables 2 and 3(A and B), castalagin showed a highest activity against HSV-1, even exceeding that of ACV. Vescalagin registered also a strong antiviral effect against HSV-1, and grandinin a markedly lower activity. In their activity against HSV-2 ellagitannins showed approximately similar antiviral effects, each of them being significantly inferior to that of ACV. These results characterize ellagitannins here investigated as prospective anti-herpesvirus agents.

The FFU reduction assay was applied for testing the combination effects of ellagitannins and ACV. The combination effects of each of the three ellagitannins with ACV were evaluated against the same HSV-1 and HSV-2 strains. From the data thus gathered were constructed 3D synergy plots (Figs. 2 and 3). As it can be seen in Fig. 2a, the combination of castalagin and ACV demonstrated a marked synergistic effect (222.06 $\mu M^2\%$) on the replication of the Victoria HSV-1 strain within a pick zone of 0.0015–0.03 $\mu M$ of castalagin and 0.33–0.66 $\mu M$ of ACV.

The results obtained in the testing of combination of vescalagin plus ACV (Fig. 2b) and those of grandinin plus ACV (Fig. 2c) also showed well-expressed synergistic effects, namely 205.09 $\mu M^2\%$ and 106 $\mu M^2\%$, respectively. In the case of vescalagin the synergistic peak zone was registered at the compound concentration of 0.009–0.018 $\mu M$ and 0.16–0.33 $\mu M$ ACV, and in the case of grandinin, at concentrations of 0.05–0.1 $\mu M$ and 0.33 $\mu M$ of ACV.

The results of the examination of the effects of combining the same three ellagitannins with ACV against the HSV-2 (Bja strain) are shown in Fig. 3. The combination of castalagin and vescalagin with ACV also demonstrated synergism, but with markedly lower values (71.62 $\mu M^2\%$ and 87.56 $\mu M^2\%$, respectively), and the magnitude of the synergistic effect with grandinin (132.78 $\mu M^2\%$) was closer to that against HSV-1.

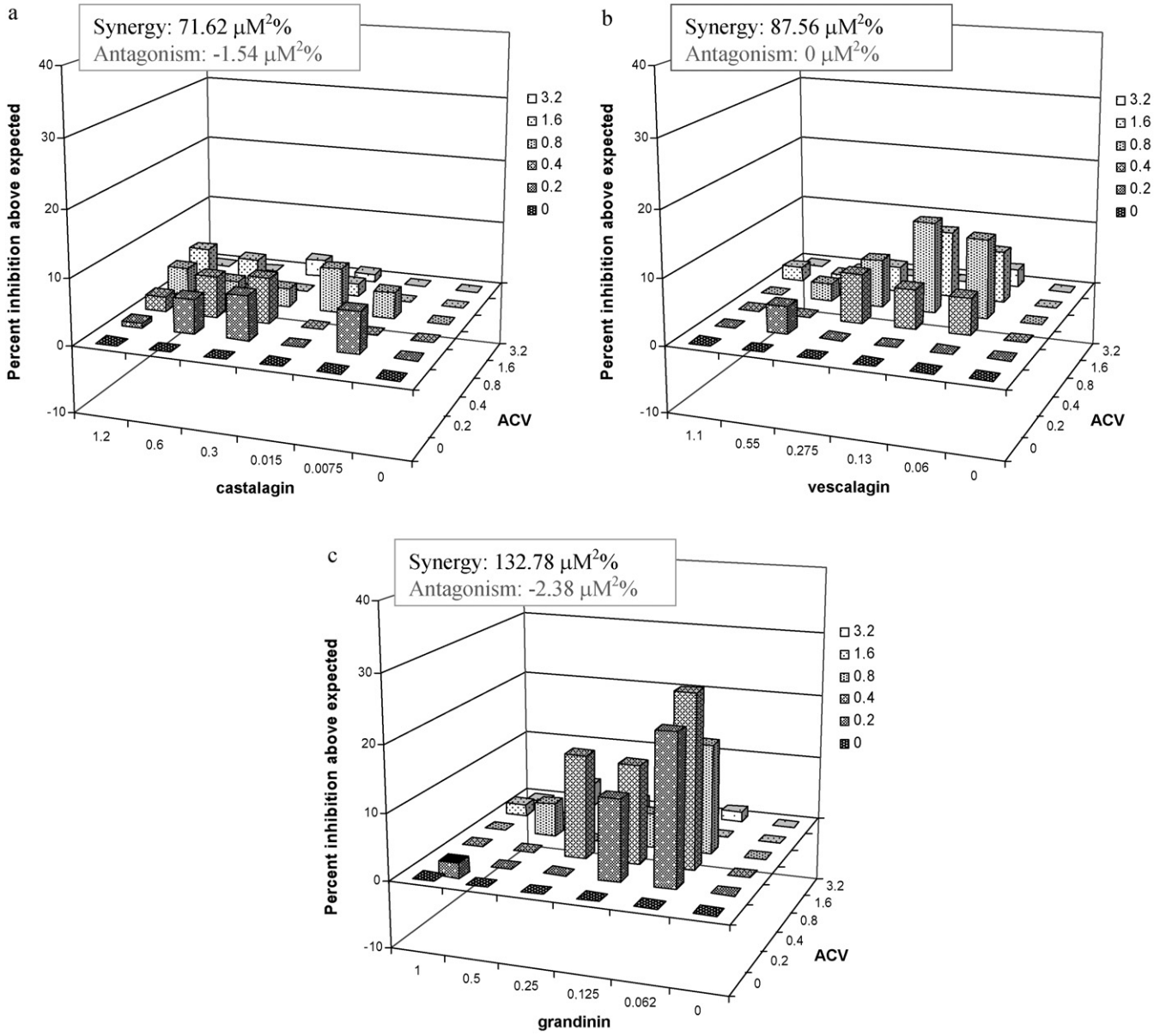


**Fig. 3.** Effect of combinations of ellagitannins and ACV on HSV-2 (Bja strain) replication: (a) castalagin and ACV; (b) vescalagin and ACV; (c) grandinin and ACV.

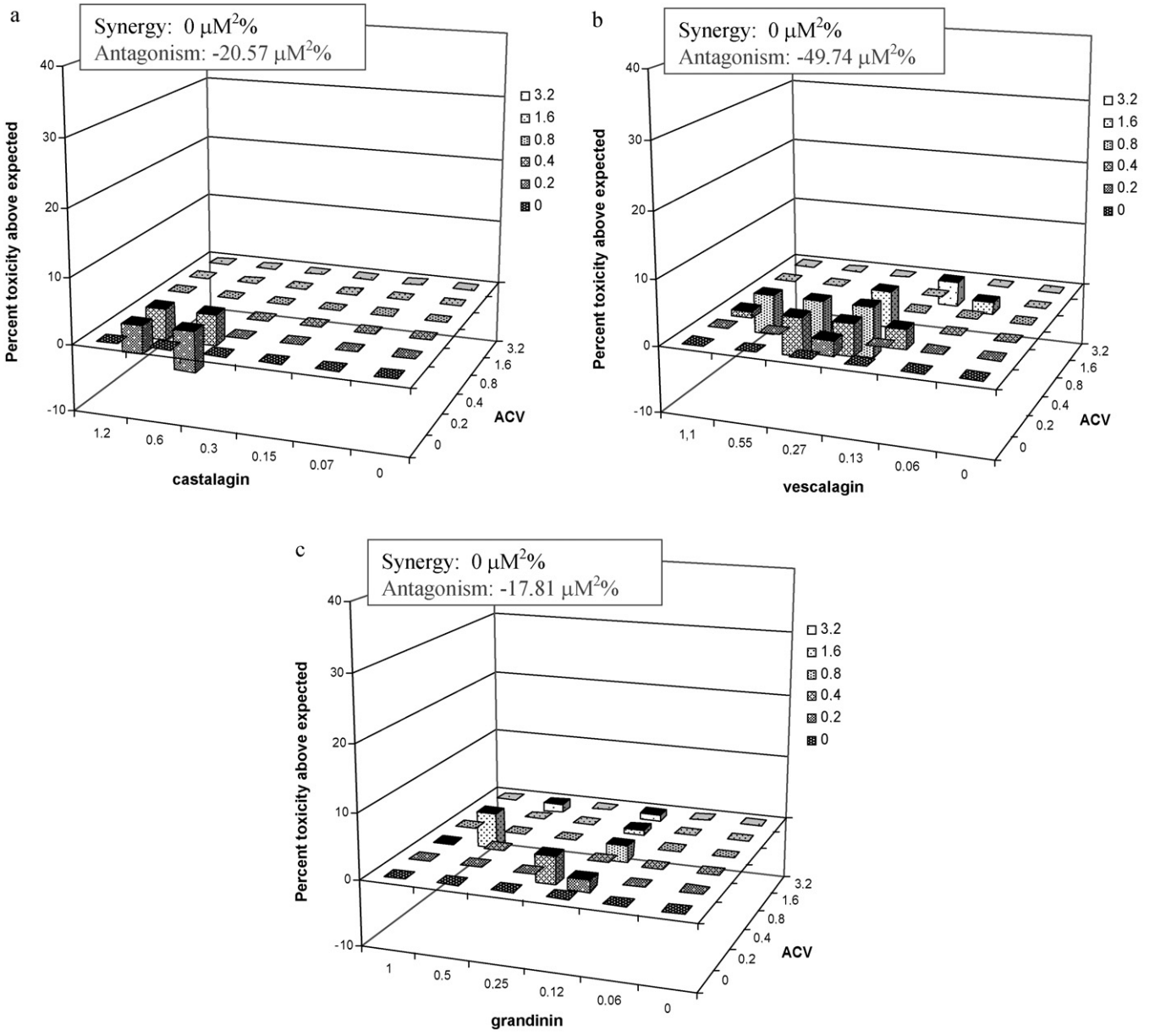


**Fig. 4.** Cytotoxicity of combinations of ellagitannins and ACV on monolayer MDBK cells: (a) castalagin and ACV; (b) vescalagin and ACV; (c) grandinin and ACV.

The combination effects of ellagitannins with ACV on intact cell monolayers (Fig. 4), evaluated by the neutral red uptake assay, demonstrated a marked antagonism and a complete lack of synergistic interactions. These data indicate that the observed synergistic combination effect of ellagitannins tested and ACV on the *in vitro* replication of HSV-1 and HSV-2 are virus-specific.

## 4. Discussion

The results obtained clearly demonstrate the synergistic character of the combination inhibitory effect of *C*-glucosidic ellagitannins such as castalagin, vescalagin or grandinin and ACV on the replication of TK(+) ACV-sensitive strains of both HSV-1 and HSV-2 in MDBK cells. Furthermore, this combination effect is markedly selective. Interestingly, another ellagitannin referred to as eugeniin, also known as tellimagrandin II, which belongs to the glucopyranosic class of these natural products, has also been shown to act synergistically with ACV against replication of the ACV-sensitive HSV-1 strain 7401H in Vero cells (Kurokawa et al., 1998, 2001). These authors have used another methodology (isobologram method) for characterizing the combination antiviral activity. As far as the individual effects of the substances evaluated herein, these *C*-glucosidic ellagitannins are significantly more active against HSV-1 than eugeniin (Kurokawa et al., 1998) and expressed an activity against HSV-2 similar to that of casuarinin, another *C*-glucosidic ellagitannin isolated from *Terminalia arjuna* Linn. (Cheng et al., 2002).

The synergistic anti-herpesvirus effects of the combinations of ellagitannins and ACV infer that ACV and ellagitannins target different molecular entities involved in the replication of herpes simplex viruses. It is known that ACV specifically targets the TK(+) herpes virus encoded DNA polymerase (Elion, 1982), but the mode of anti-herpetic action of the studied ellagitannins has yet to be elucidated. It was found that the anti-herpesvirus activity (HSV-1, HSV-2) of the glucopyranosic ellagitannin eugeniin implicates an inhibition of both the viral DNA polymerase activity and late protein synthesis (Kurokawa et al., 1998). Moreover, cowaniin, another *C*-glucosidic ellagitannin, isolated from *Cowania mexicana* (*Rosaceae*)

**Table 3**
Antiviral effect selectivity of ellagitannins and ACV against the replication of HSV-1 (Victoria strain) and HSV-2 (Bja strain).

(A) In monolayer cell culture (neutral red uptake)

| Compound | SI[a] | | | |
|---|---|---|---|---|
| | HSV-1 | | HSV-2 | |
| | FFU reduction test | CPE inhibition test | FFU reduction test | CPE inhibition test |
| Castalagin | 4498.0 | 930.0 | 89.9 | 61.3 |
| Vescalagin | 909.0 | 378.7 | 123.9 | 72.5 |
| Grandinin | 88.7 | 40.4 | 71.0 | 45.5 |
| ACV | 972.8 | 881.6 | 810.5 | 744.8 |

(B) In growing cells

| Compound | SI[b] | | | | SI[c] | | | |
|---|---|---|---|---|---|---|---|---|
| | HSV-1 | | HSV-2 | | HSV-1 | | HSV-2 | |
| | FFU reduction test | CPE inhibition test | FFU reduction test | CPE inhibition test | FFU reduction test | CPE inhibition test | FFU reduction test | CPE inhibition test |
| Castalagin | 885.0 | 183.1 | 17.7 | 12.1 | 1113.3 | 230.4 | 22.3 | 15.2 |
| Vescalagin | 257.6 | 107.3 | 35.1 | 20.6 | 253.3 | 105.6 | 34.6 | 20.2 |
| Grandinin | 13.5 | 6.1 | 10.8 | 6.9 | 22.1 | 10.1 | 17.7 | 11.3 |
| ACV | 285.7 | 285.5 | 237.5 | 218.4 | 310.5 | 280.9 | 258.1 | 237.4 |

[a] Selectivity index (SI) = $CC_{50}/IC_{50}$.
[b] $CGIC_{50}$ evaluated by cell counting test.
[c] $CGIC_{50}$ evaluated by neutral red uptake.

demonstrated an inhibitory effect on activation of the Epstein-Barr virus early antigen (Ito et al., 2007). The aforementioned ellagitannin casuarinin exerts its effect on HSV-2 replication by preventing the virus attachment to the host-cell and the viral penetration, and also disturbing the late event(s) of the growth cycle. Besides, this compound possesses a strong virucidal effect (Cheng et al., 2002). These data clearly show that the ellagitannins we evaluated here and related compounds might constitute interesting anti-herpetic agents by virtue of the their capability to perturbe some stages in the herpesvirus replication, but the capability of ellagitannins to penetrate cell membranes still need to be ascertained. Moreover, a putative virucidal effect known for tannins could be suspected as a component of the antiviral activity of ellagitannins tested and that imposes further elucidation. More and more data have been accumulated as concern the chemotherapeutic perspectives of substances with virucidal potential (Kaptein et al., 2008; Harden et al., 2008).

Evidently, elucidation of the mode of antiviral action of castalagin, vescalagin and grandinin merits a special attention and will be the focus of future studies. Nevertheless, the data we collected here on their activity demonstrate the high potential of these *C*-glucosidic ellagitannins as antiherpetic agents, which could be advantageously combined with ACV, and strongly underline that the family of polyphenolic natural products to which they belong deserves further attention as a source of chemotherapeutic drugs possibly applicable against herpesvirus infections.

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