A Cell Type-specific and Gap Junction-independent Mechanism for the Herpes Simplex Virus-1 Thymidine Kinase Gene/Ganciclovir-mediated Bystander Effect

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ABSTRACT

Tumor cells expressing the herpes simplex virus type 1 thymidine kinase (HSV-tk) gene are killed by nucleoside analogues such as ganciclovir (GCV). GCV affects not only the cells expressing HSV-tk but also neighboring cells that do not express the gene; this phenomenon commonly is called “bystander effect.” GCV metabolites transfer via gap junctional intercellular communication (GJIC) accounts for the bystander effect in different cell lines, but other mechanisms have also been described. In this study, we analyzed the mechanisms of the bystander effect in two cell lines exhibiting different capacities of communication (DHD/K12 and 9L). The 9L cells exhibited a very good bystander effect, which was completely blocked by a long-term inhibitor of GJIC, 18 α-glycyrrhetinic acid. DHD/K12 cells exhibited a moderate bystander effect that was not abolished by 18 α-glycyrrhetinic acid or 1-octanol, another strong inhibitor of GJIC. Interestingly, we also observed a bystander effect in cultures where HSV-tk-expressing DHD/K12 cells were physically separated from their untransfected counterparts but grown in the same medium. Moreover, the transfer of filtered conditioned medium from GCV-treated HSV-tk-expressing DHD/K12 cells to DHD/K12 parental cells induced a decrease of survival in a concentration-dependent manner, suggesting that the bystander effect in this cell line was mediated by a soluble factor.

INTRODUCTION

The transfer of suicide genes into tumor cells constitutes a novel approach for the treatment of human cancers. The HSV-tk gene has been used successfully to eradicate a variety of tumors in animal models (1–8) and has also been evaluated in several clinical protocols (9, 10). This strategy takes advantage of HSV-tk capacity to phosphorylate nucleoside analogues such as GCV into GCV-monophosphate. Subsequent phosphorylation of GCV-monophosphate by cellular kinases leads to the formation of cytotoxic GCV-triphosphate, which is incorporated into DNA and inhibits DNA polymerase (11–13). Interestingly, untransduced adjacent cells are also sensitive to GCV treatment. Therefore, transduction of a limited number of cells with the suicide gene could be sufficient to eradicate a tumor (5, 7, 14). This phenomenon has been called “bystander effect.”

Several mechanisms have been proposed to explain the bystander effect. It could be linked to the level of TK activity in transduced cells (15) or to the uptake of apoptotic vesicles by the adjacent nontransduced cells (16). Moreover, the transfer of GCV-triphosphate through gap junctions plays an important role in this mechanism both in vitro and in vivo (17–22). Finally, other phenomena such as immune reaction (23, 24) or blood vessel destruction (25) seem to also be involved in the bystander effect in vivo.

In the present work, we studied the mechanisms of the bystander effect in vitro in two cell lines: a rat colon adenocarcinoma cell line (DHD/K12) and a rat gliosarcoma cell line (9L). These cell lines exhibit different capacities of communication and have been used as targets for in vivo HSV-tk gene therapy studies (5, 25, 26). We showed that GJIC was essential for the bystander effect in 9L cells, as was described previously in numerous cell lines, but not in DHD/K12 cells where the release of soluble factors by the HSV-tk-expressing GCV-treated cells seems to play an important role.

MATERIALS AND METHODS

Cell Lines and Reagents

Rat colon adenocarcinoma DHD/K12/Prob cells were grown in DMEM medium (Life Technologies, Gaithersburg, MD) supplemented with 5% FBS

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3 The abbreviations used are: HSV-tk, herpes simplex virus type 1 thymidine kinase gene; GCV, ganciclovir; TK, thymidine kinase; GJIC, gap junctional intercellular communication; FBS, fetal bovine serum; AGA, 18-α-glycyrrhetinic acid.
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were pretreated for 5 days with 1 mM 1-octanol. AGA and 1-octanol were obtained from Sigma-Aldrich (St. Louis, MO). Twenty-five microliters of 1000× AGA and 1-octanol were added to the medium.

These cocultures were grown for 7 days (DHD/K12) or 4 days (9L). After the treatment, the HSV-\(^\text{tk}\)– cells in the 24-well plate were harvested, and the total number of living cells was determined by the trypan blue exclusion method and compared to HSV-\(^\text{tk}\)– DHD/K12 cells, grown separately.

The medium was changed every 2 days. Coverslips were then placed in the perfusion chamber of a Zeiss microscope and perfused with EA01 buffer (137 mM NaCl, 5.7 mM KCl, 1.8 mM CaCl\(_2\), 22.2 mM d-Glucose, 10 mM HEPES). A single cell in a confluent area was microinjected with Lucifer yellow dye. The total number of colored adjacent cells was counted 60 s after the end of the injection.

Separated Culture Experiments. HSV-\(^\text{tk}\)-expressing cells (2 × 10\(^5\) for DHD/K12-TK and 1 × 10\(^7\) for 9L-TK) were seeded on a matrigel-precoated milliplate (12 mm diameter; 0.4 μm pore size; Millipore Corporation, Bedford, MA). The milliplates were placed in 24-well plates. Untransduced DHD/K12 (10\(^5\) cells) or 9L (5 × 10\(^5\) cells) were seeded in the wells. The two populations of cells (HSV-\(^\text{tk}\)+ cells and HSV-\(^\text{tk}\)– cells) were incubated in culture medium and seeded at low density on polyornithine coated glass coverslips. The cells were incubated in test medium and grown to confluence for 5–6 days. The medium was changed every 2 days. Coverslips were then placed in the perfusion chamber of a Zeiss microscopy and perfused with EA01 buffer (137 mM NaCl, 5.7 mM KCl, 1.8 mM CaCl\(_2\), 22.2 mM d-Glucose, 10 mM HEPES). A single cell in a confluent area was microinjected with Lucifer yellow dye (Sigma; 5% w/v in 0.1 M LiCl) by passing 0.5 Hz/500 ms-1 mA hyperpolarizing current pulses for 30 s through the electrode. The total number of colored adjacent cells was counted 60 s after the end of the injection.

**Medium Transfer.** HSV-\(^\text{tk}\)-expressing DHD/K12 cells were grown in 24-well plates (2 × 10\(^5\) cells/well) in different volumes of medium (300, 500, 700, and 1000 μl) containing or not (control) 20 μM GCV. Twenty-four h later, the medium was collected, filtered on a Sartorius Minisart (pore size, 0.2 μm; Sartorius AG, Göttingen, Germany) and transferred into wells containing untransduced DHD/K12 cells (10\(^4\) cells/well; 24-well plate). This operation was repeated daily for 7 days. After the treatment, the HSV-\(^\text{tk}\)– cells in the 24-well plates were harvested, and the total number of living cells was determined by the trypan blue exclusion method and compared to HSV-\(^\text{tk}\)– control cells, grown in separate wells.

### Table 1: Comparison of the bystander effect in DHD/K12 (A) and 9L (B) cell lines.

<table>
<thead>
<tr>
<th>% of DHD/K12-TK cells</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Survival (%)</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
</tr>
</tbody>
</table>

**Fig. 1** Comparison of the bystander effect in DHD/K12 (A) and 9L (B) cell lines. HSV-\(^\text{tk}\)+ and HSV-\(^\text{tk}\)– cells were mixed in different ratios in 96-well plates. These cocultures were treated with 20 μM GCV alone ([■]), 20 μM GCV + 70 μM AGA ([○]), or 20 μM GCV + 1 mM 1-octanol ([△]). These cocultures were grown for 7 days (DHD/K12) or 4 days (9L). Cell viability was measured with the WST-1 test. Statistical analyses were performed in all culture conditions for the two cell lines. AGA and 1-octanol did not block the bystander effect in DHD/K12 cells. AGA blocked the bystander effect in the 9L cells at all ratios of HSV-\(^\text{tk}\)+ and HSV-\(^\text{tk}\)– cells (\(P < 0.05\); ANOVA, Tukey post-tests).

(20% FBS in the experiment described in Fig. 1), 1% 200 mM glutamine, 1% 1x HEPES, 1% 0.55 mM arginine, and 1% 5 mg/ml penicillin-streptomycin. Rat gliosarcoma 9L cells were grown in RPMI 1640 (Life Technologies) supplemented with 10% FBS, 1% nonessential amino acids, 1% 100 μM sodium pyruvate, and 1% 5 mg/ml penicillin-streptomycin. Cells were grown at 37°C in 5% CO\(_2\).

9L-TK cells infected with a Moloney murine leukemia virus retrovirus containing the HSV-\(^\text{tk}\) gene driven by an SV40 promoter were obtained from C. Grignet (Laboratory of Virology, University of Liège, Belgium). The DHD/K12-TK cell line was generated by infection with the same retrovirus (26).

AGA and 1-octanol were obtained from Sigma-Aldrich (St. Louis, MO). AGA was dissolved in DMSO.

**Estimation of the Bystander Effect.** To test the bystander effect, various proportions of HSV-\(^\text{tk}\)-expressing cells were mixed (100, 50, 25, 15, 10, 5, and 0%) with corresponding untransduced cells. These cocultures were then treated daily with 20 μM GCV (Roche S.A., Brussels, Belgium) for 7 days (DHD/K12) or 4 days (9L). At the end of the treatment, cell viability was tested with WST-1 proliferation reagent as recommended by the manufacturer (Boehringer Mannheim, Mannheim, Germany). During the GCV treatment, 70 μM AGA (9L cells) and 70 μM AGA or 1 mM 1-octanol (DHD/K12 cells) were added to the medium.

Before the 1-octanol bystander experiment, DHD/K12 cells were pretreated for 5 days with 1 mM 1-octanol.

**Dye Transfer.** GJIC was assessed by dye-transfer assay using Lucifer yellow as a tracer according to Stewart (27). Briefly, cells were harvested in culture medium and seeded at low density on polyornithine coated glass coverslips. The cells were incubated in test medium and grown to confluence for 5–6 days. The medium was changed every 2 days. Coverslips were then placed in the perfusion chamber of a Zeiss microscope and perfused with EA01 buffer (137 mM NaCl, 5.7 mM KCl, 1.8 mM CaCl\(_2\), 22.2 mM d-Glucose, 10 mM HEPES). A single cell in a confluent area was microinjected with Lucifer yellow dye (Sigma; 5% w/v in 0.1 M LiCl) by passing 0.5 Hz/500 ms-1 mA hyperpolarizing current pulses for 30 s through the electrode. The total number of colored adjacent cells was counted 60 s after the end of the injection.

Separation of cell lines was performed in all culture conditions for the two cell lines. AGA and 1-octanol did not block the bystander effect in DHD/K12 cells. AGA blocked the bystander effect in the 9L cells at all ratios of HSV-\(^\text{tk}\)+ and HSV-\(^\text{tk}\)– cells (\(P < 0.05\); ANOVA, Tukey post-tests).
Statistical Analysis. All statistical analyses were performed using a commercially available software (GraphPad Software Incorporation, San Diego, CA). Cell survival experiments involving 9L and DHD/K12 cells were analyzed using a parametric unpaired two-way ANOVA with Tukey post-tests. Dye-transfer experiments were compared using a nonparametric two-way ANOVA with Dunn’s post-tests for both DHD/K12 and 9L cells because it appeared that the results did not fit a gaussian distribution. Differences were considered significant when the probability (P) was <0.05.

RESULTS

Analysis of the Bystander Effect in DHD/K12 and 9L Cell Lines. We compared the extent of the bystander effect in 9L and DHD/K12 cells. In these experiments HSV-tk-expressing cells (HSV-tk+) and parental cells (HSV-tk−) were mixed at different ratios and treated with 20 μM GCV.

The bystander effect varied among the two cell lines (Fig. 1). 9L cells showed the highest bystander effect; in the presence of 5% HSV-tk+ cells, 52% of the cells were killed by the GCV treatment. DHD/K12 cells showed only a moderate bystander effect; 5 or 10% HSV-tk+ cells led to the killing of 15 or 40% of the cells, respectively. Because 9L cells were treated only for 4 days, a higher proportion of the cells were still viable at the end of the experiment when 100% TK+ cells were considered. Longer treatments led to a complete eradication of the TK+ 9L cells (data not shown).

To examine whether GJIC was responsible for the bystander effect observed in DHD/K12 and 9L cells, GCV treatments were performed in the presence of AGA, a selective and reversible inhibitor of GJIC (Fig. 1; Ref. 28). AGA alone did not have any toxicity on the different cell lines (data not shown). We observed that 70 μM AGA completely inhibited the bystander effect in 9L cells but not in DHD/K12 cells. To confirm these results, GCV treatments of DHD/K12 cells were also performed in the presence of 1-octanol, another selective and reversible inhibitor of cell-cell communication via gap junction (Fig. 1A; Refs. 29–31). 1-Octanol alone did not have any toxicity on DHD/K12 cells, but it had a strong cytotoxic effect, even at low concentrations, on 9L cells (data not shown). We observed that 1-octanol, like AGA, did not significantly inhibit the bystander effect in DHD/K12 cells.

These results suggested that the bystander effect was related to GJIC in 9L cells but not in DHD/K12 cells.

Effect of AGA and 1-Octanol on Intercellular Communications in DHD/K12 and 9L Cell Lines. The effect of AGA on GJIC in DHD/K12 and 9L cells and of 1-octanol on GJIC in DHD/K12 cells was further studied by dye transfer. Cells were grown to confluence. Lucifer yellow was injected in a single cell, and the number of dye-colored cells was counted 60 s later. These experiments demonstrated that 9L cells communicated very efficiently because the injection of a single cell allowed dye transfer to an average of 102.1 cells. Conversely, DHD/K12 cells communicated poorly, the dye being transferred to 3.09 cells (Table 1).

In both cell lines, AGA treatment significantly inhibited the number of dye-coupled cells per microinjection (3.4 communicating cells after AGA treatment in 9L cells and 1.05 after AGA treatment in DHD/K12 cells). In DHD/K12 cells, 1-octanol also significantly inhibited the number of dye-coupled cells (0.64 communicating cells after 1-octanol treatment; Table 1).

These data confirmed that the bystander effect was dependent on GJIC in 9L cells because it was blocked by AGA. Furthermore, the bystander effect in DHD/K12 cells was independent of GJIC because it was not affected by AGA or 1-octanol treatments. It should however be noted that the constitutive intercellular communication was much lower in DHD/K12 cells compared with that observed in 9L cells. This DHD/K12 GJIC might be too low to allow significant transfer of GCV metabolites, indicating that another mechanism is probably responsible for the bystander effect in these cells.

Role of Soluble Factors in the Bystander Effect in DHD/K12 and 9L Cell Lines. We investigated whether soluble factors produced by dying HSV-tk+ cells might play a role in the bystander effect in DHD/K12 and 9L cells. HSV-tk− cells were first cocultivated with HSV-tk+ cells in the same medium but separated by a membrane filter as described in “Materials and Methods” (Fig. 2). Under these conditions, GCV treatment induced the killing of 77.5% of HSV-tk− DHD/K12 cells compared with control untreated cells. However, in similar conditions, we did not observe any toxicity on HSV-tk− 9L cells (Fig. 2) or on HSV-tk− C6 cells (data not shown). These results showed that cell-cell contacts are required for bystander killing in 9L cells but not in DHD/K12 cells. The bystander effect in this cell line was probably due to the release of soluble factors from the dying HSV-tk+ cells in the medium.

To confirm this observation, separate experiments were performed on DHD/K12 cells. HSV-tk+ DHD/K12 cells were grown in different volumes of medium (300, 500, 700, and 1000 μl) and treated with GCV. Conditioned medium from HSV-tk+ GCV-treated DHD/K12 cells was filtered and then transferred onto HSV-tk− DHD/K12 cells. The transfer of 300 μl of conditioned medium induced the killing of 69.3% of HSV-tk− DHD/K12 cells (Fig. 3), whereas filtered medium from untreated cells did not induce any cell death. Moreover, the toxicity of conditioned medium decreased when the volume increased (e.g., transfer of 700 μl of conditioned medium led to the killing of only 35.8% of HSV-tk− DHD/K12 cells). These results show that the cytotoxicity of the soluble factors contained in the conditioned medium was related to their concen-

Table 1  Gap junctional communication after microinjection of Lucifer yellow

<table>
<thead>
<tr>
<th>Cells</th>
<th>Number of communicating cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHD/K12 control</td>
<td>3.09 (± 1.3)</td>
</tr>
<tr>
<td>DHD/K12 + AGA</td>
<td>1.05 (± 1.7)</td>
</tr>
<tr>
<td>DHD/K12 + 1-octanol</td>
<td>0.64 (± 0.93)</td>
</tr>
<tr>
<td>9L control</td>
<td>102.1 (± 82.8)</td>
</tr>
<tr>
<td>9L + AGA</td>
<td>3.4 (± 5.8)</td>
</tr>
</tbody>
</table>
Gap Junction-independent Mechanism for the Bystander Effect

The differences between the GCV-treated and untreated or treated with GCV (20 μM) medium were replaced every day for 7 days (DHD/K12) or 4 days (9L). After treatment, survival was assessed by the trypan blue exclusion method and compared to the untreated DHD/K12 cell death in a concentration-dependent manner. Different volumes (300, 500, 700, or 1000 μl) of filtered (0.2 μm pore size) medium from 2 × 10⁶ HSV-tk+ GCV-treated or untreated (control) cells were transferred into wells containing 10⁵ HSV-tk− cells. This operation was repeated daily for 7 days. After GCV treatment, survival was assessed by the trypan blue exclusion method and compared to the cells cultivated in normal, unconditioned medium (100%). Each column represents the mean of at least six independent experiments (bars, SD). The differences between the GCV-treated and untreated DHD/K12 cells were significant (P < 0.001; ANOVA parametric Tukey post-tests).

Fig. 3 Medium from GCV-treated HSV-tk+ DHD/K12 cells induced HSV-tk− DHD/K12 cell death in a concentration-dependent manner. Different volumes (300, 500, 700, or 1000 μl) of filtered (0.2 μm pore size) medium from 2 × 10⁶ HSV-tk+ GCV-treated or untreated (control) cells were transferred into wells containing 10⁵ HSV-tk− cells. This operation was repeated daily for 7 days. After GCV treatment, survival was assessed by the trypan blue exclusion method and compared to the cells cultivated in normal, unconditioned medium (100%). Each column represents the mean of at least six independent experiments (bars, SD). The P values from the ANOVA parametric Tukey post-tests are indicated.

AGA and 1-octanol did not significantly inhibit the bystander effect in DHD/K12 cells, although they significantly inhibited gap junctional communication, suggesting that GJIC was not involved in this process. Separation of HSV-tk+ and HSV-tk− DHD/K12 cells by a filter membrane led the killing of HSV-tk− cells after GCV treatment. Moreover, the transfer of different volumes of filtered conditioned medium from HSV-tk+ GCV-treated cells to HSV-tk− cells also killed these cells in a concentration-dependent manner. These data indicated that direct intercellular contacts was not required for the bystander effect in DHD/K12 cells. This phenomenon could be explained by the phagocytosis by HSV-tk− cells of apoptotic vesicles released in the medium by dying HSV-tk+ cells as has been described by Freeman et al. (16) or by the transmission of soluble factors. However, the filtration of conditioned medium through 0.2 μm filters probably retained apoptotic bodies and thus supported the hypothesis of soluble factors mediating the bystander effect in DHD/K12 cells.

Our report as well as other reports indicate that the mechanism of the bystander effect is cell type specific. A recent report showed that the inhibition of GJIC by 1-octanol in lung cancer cell lines did not reduce the tumor cell killing through the bystander effect. However, the authors failed to detect any bystander effect after physical separation of HSV-tk+ and HSV-tk− cells, indicating that this effect still required intercellular contacts (29). Different studies showed that phosphorylated GCV cannot pass through the cell membranes (17), but it was also reported that rat hepatoma cells, which express connexin 43, were able to take up membrane-impermeable dye from the culture medium through connexon hemichannels that did not.

Fig. 2 Bystander effect between separated HSV-tk+ and HSV-tk− cells. HSV-tk− cells were plated in 24-well plates (10⁶ DHD/K12 cells and 5 × 10⁵ 9L cells per well), and HSV-tk+ cells were seeded on a matrigel-precocated millicell (0.4 μm pore size) positioned in the same well over the HSV-tk− cells. The two populations were thus separated in the same well and cultivated in the same medium. Cells were untreated or treated with GCV (20 μM). The differences between the GCV-treated and untreated or treated with GCV (20 μM) medium were replaced every day for 7 days (DHD/K12) or 4 days (9L). After treatment, survival was assessed by the trypan blue exclusion method and compared to the controls. Each column represents the mean of at least six independent experiments (bars, SD). The differences between the GCV-treated and untreated DHD/K12 cells were significant (P < 0.001; ANOVA parametric Tukey post-tests).

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align with hemichannels from neighboring cells to form functional GJIC (32). Another group reported that the bystander effect was not correlated with gap junctional communication in two human colon adenocarcinoma cell lines (SW620 and HT-29) and that GCV nucleotides can be transferred from HSV-tk-expressing to nonexpressing cells in cocultures even in cell lines exhibiting low levels of GJIC. If functional hemichannels were responsible for the uptake of GCV nucleotides from the extracellular environment, this mechanism would depend on the proximity of neighboring cells because extracellular nucleotides would be rapidly diluted in the medium or degraded if the cells were too far apart (33). In contrast, our results suggested that cellular contacts or cellular proximity were not required for bystander effect in DHD/K12 cells and provide strong evidence for the existence of a novel mechanism: the release of soluble factors. The identification of these soluble factors will require additional experiments.

We think that multiple pathways rather than a single pathway may be involved in the mechanism of bystander effect in different cell types. Clearly, GJIC plays a crucial role in the bystander effect in numerous cell types as shown in many reports. Therefore, some studies investigated the possibility of enhancement of the bystander effect, in vitro and in vivo, by pharmacological modulation of gap junctions (34, 35). But as we and other reported, it is obvious that other mechanisms are also involved in distinct cell types. The understanding of these mechanisms will be a prerequisite to the development of new pharmacological tools aimed at the modulation of the bystander effect.

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REFERENCES


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