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Cell Killing by Simian Virus 40: Protective Effect of Chloroquine

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Treatment of CV-1 cells with chloroquine before infection by simian virus 40 resulted in the accumulation of fewer nonviable, trypan blue-stainable cells at 72 h. The drug did not affect the fraction of infected T-antigen-producing cells or the viral yields. It did diminish the apparent redistribution of lysosomal *N*-acetyl-β-glucosaminidase from a particulate to a soluble cell fraction, and it caused an increase in the size and number of lysosomes.

If lysosomal processes play a role in the sequence of events leading to cell killing by viruses (1), then it might be expected that substances which affect lysosomal processes might also affect viral cytopathology. For this reason we have examined the effect of chloroquine on simian virus 40 (SV40) cytopathology.

Cells exposed to chloroquine rapidly take up and concentrate this weak base in their lysosomes, where it is trapped by protonation (2). At the concentration of chloroquine attained in lysosomes, this drug severely affects the activity in vitro of cathespin B_1 (12), a lysosomal protease believed to be important in the initiation of proteolysis (5). The degradation of endogenous protein is also inhibited in chloroquine-treated cells (12). In addition, it was reported that chloroquine stabilizes lysosomal membranes in vitro (10).

The results in Table 1 show that chloroquine treatment inhibited cell killing by SV40, as indicated by reduced levels of nonviable trypan blue-stainable cells at 72 h. In contrast, chloroquine treatment did not affect the fraction of infected cells, as indicated by indirect immunofluorescent staining for the SV40 T antigen, an early viral gene product. In untreated infected cultures, the fraction of infected T-antigen-pro-

TABLE 1. *Protective effect of chloroquine*

Cultural conditions[a]		% Trypan blue-stainable cells at 72 h[b]	% T-antigen-containing nuclei at 48 h[b]	Viral yields at 72 h (PFU/ml)[c]	% *N*-acetyl-β-glucosaminidase in soluble cell fraction at 48 h[d]	Activity (%) in media at 48 h[e]	
Chloroquine	Virus					*N*-acetyl-β-glucosaminidase	Lactic acid dehydrogenase
−	+	93	95	3×10^7	54 ± 1	54 ± 1	54 ± 1
−	−	2	0		40 ± 1	34 ± 2	18 ± 1
+	+	48	90	5×10^7	44 ± 6	35 ± 2	40 ± 1
+	−	2	0		33 ± 1	33 ± 1	24 ± 1

[a] Chloroquine diphosphate (60 μg/ml) was added to monolayer cultures of CV-1 green monkey kidney cells for 6 h (7). Cultures were then washed with medium, infected as indicated with SV40 strain 777 at a multiplicity of infection of 10 plaque-forming units/cell, and, after adsorption for 2 h at 37°C, refed with normal medium. Samples that were tested for enzyme release into the medium were washed and refed with serum-free medium after infection.

[b] Nonviable trypan blue-stainable cells and indirect immunofluorescent staining for SV40 T antigen were as previously described (9).

[c] PFU, Plaque-forming units.

[d] Percent activity in washed cells in the soluble cell fraction. Cell fractionation and enzyme assay were as previously described (9). Results are the average of duplicate experiments ± the difference between the individual experiments and their mean.

[e] Whole cells and the serum-free overlay medium were harvested and assayed for enzyme activity seperately. Enzyme assays were as previously described (9). Results are the average of duplicate experiments ± the difference between the individual experiments and their mean.

ducing cells at 48 h was quantitatively similar to the fraction of nonviable trypan blue-stainable cells at 72 h, in agreement with our earlier findings (9). Chloroquine treatment reduced the fraction of trypan blue-stainable cells by about half, while not affecting the fraction of infected T-antigen-producing cells. Viral replication was also not affected by chloroquine treatment. In some experiments, cell-associated and extracellular virus were titrated separately at various times after infection. Chloroquine had little, if any, effect on virus release.

Results in Table 1 show that SV40 infection of CV-1 cells resulted in a redistribution of the lysosomal enzyme *N*-acetyl-β-glucosaminidase (EC 3.2.1.30) from a particulate to a soluble cell fraction, in agreement with our earlier report (9). However, both chloroquine-treated infected and uninfected cells showed a reduced level of lysosomal enzyme redistribution compared with the corresponding untreated cultures.

Results in Table 1 also show that SV40 infection of CV-1 cells resulted in the release into the media of lysosomal *N*-acetyl-β-glucosaminidase and of cytoplasmic lactic acid dehydrogenase (EC 1.1.1.27), in agreement with our earlier findings (8, 9). The chloroquine treatment diminished the release of both enzymes from the infected cultures.

Chloroquine treatment caused some alteration of the appearance of lysosomes stained with acridine orange dye (Fig. 1). Both control and

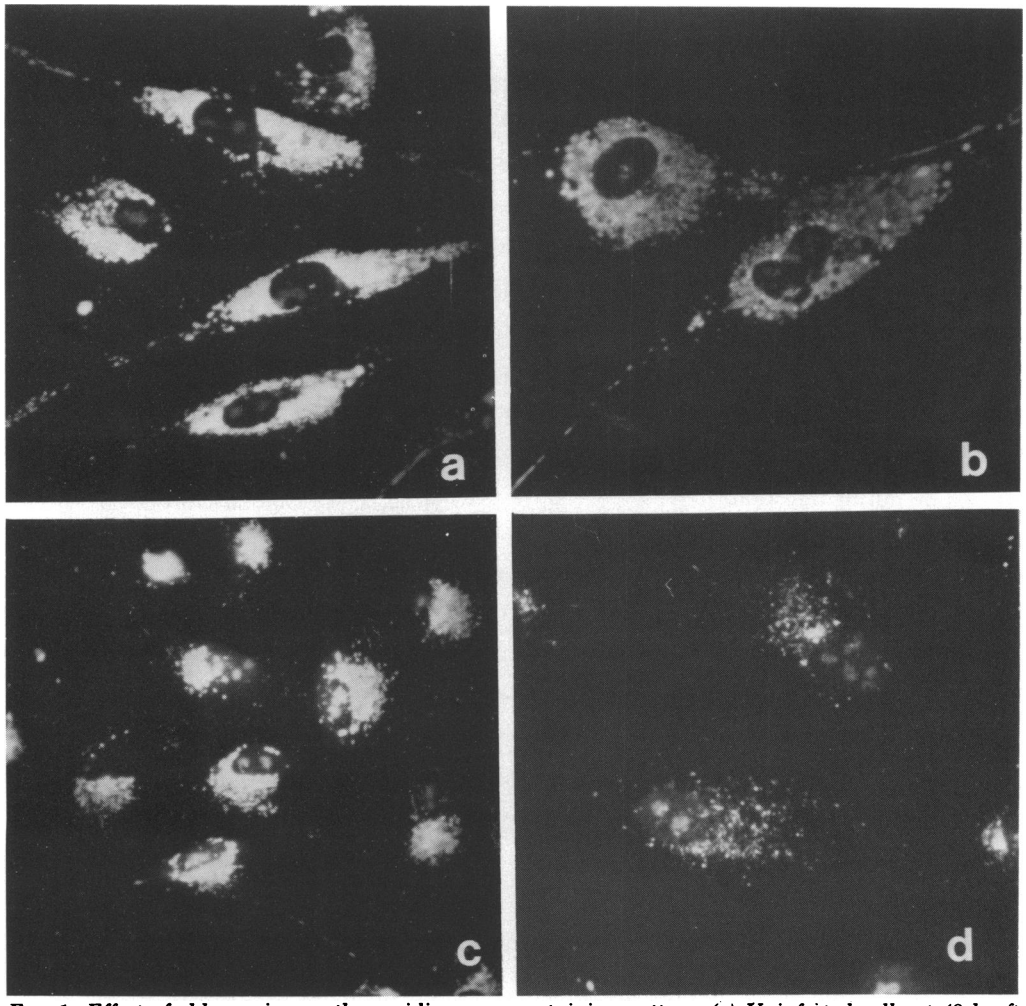

FIG. 1. *Effect of chloroquine on the acridine orange staining pattern. (a) Uninfected cells at 48 h after chloroquine treatment; (b) chloroquine-treated infected cells at 48 h; (c) untreated uninfected cells; (d) untreated infected cells at 48 h. All plates were photographed at the same magnification.*

infected CV-1 cells, when treated with chloroquine, had increased numbers of acridine orange-stained particles, and these stained particles were slightly swollen compared with those in the corresponding untreated cultures. Grossly swollen acridine orange-stained particles, which were seen in some of the untreated infected cells (data not shown), were not seen in the treated infected cultures.

The results reported here are consistent with models of viral cytopathology involving lysosomes. Nevertheless, the effects of agents which stabilize or labilize lysosomes must be interpreted with great caution. The functions of lysosomes are varied, and, therefore, depending on the conditions, these agents might affect the lysosomal system in several ways. In addition, these agents might affect other classes of cellular membranes and processes in addition to those of lysosomes. For example, Wilson (13) reported that chloroquine protects chick cells from Newcastle disease virus-induced breakdown of cellular RNA and from inhibition of cellular protein synthesis, but does not prevent lysosomal enzyme release. Furthermore, recent studies, to be reported elsewhere, suggest that lysosomes remain as intact structures during the antemortem stage of lytic SV40 infection. Consequently, we believe that the protective effect of chloroquine reported here does not result primarily from its ability to stabilize lysosomes during infection. The increased latency of the lysosomal hydrolase *N*-acetyl-β-glucosaminidase in the chloroquine-treated cultures might reflect an increased resistance of the lysosomes to breakage during the preparation of cell fractions, rather than stabilization during infection.

It might be noted that cortisone, another lysosomal stabilizer (11), had no protective effect on SV40-infected CV-1 cells (data not shown) under conditions in which this drug protects human cells from poliovirus cytopathic effects (6). In contrast, vitamin A, which is considered to be a lysosomal labilizer, did protect SV40-infected CV-1 cells in some experiments (data not shown).

The protective effect of chloroquine might be explained by the ability of this drug to induce autophagic activity (4). Autophagy may represent a cellular survival mechanism in response to pathological injury, acting to isolate damaged regions from the rest of the cell (3). The increase in the number and size of the acridine orange-stained particles in response to chloroquine treatment is consistent with this suggestion.

Regardless of the mechanism(s) by which chloroquine protects SV40-infected CV-1 cells, this drug does not affect SV40 replication. In an earlier report (9), we showed that different simian cell lines are killed at markedly different rates by SV40 despite the fact that viral replication and release are similar in each of them. The partial dissociation of cell killing from viral replication reported here and in our earlier study implies that cell killing by SV40 is not simply due to mechanical damage resulting from viral replication and/or release. Furthermore, virus production and release are not dependent on the full development of the accompanying cytopathic effects.

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