Chloroquine: Protection Against Virus-induced Cell Damage Without Inhibition of Virus Growth

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The RNA in chick embryo fibroblasts is degraded after infection of the cells by Newcastle disease virus (NDV) (Huo & Wilson, 1969). The nucleases which cause this degradation may have been located in lysosomes which break down following virus infection, as is the case in poliovirus infected KB cells (Flanagan, 1966). This latter observation suggested that the NDV induced degradation of cell RNA might be prevented by treating virus infected cells with an agent which stabilizes lysosomes. This hypothesis was examined using chloroquine diphosphate which has been reported to stabilize lysosomes in some cell types (Weissman, 1964) and to cause lysosomal breakdown in other cell types (Fedorko, Hirsch & Cohn, 1968). It was found that chloroquine did in fact protect the RNA of NDV infected cells, but not because it stabilized the lysosomes in these cells.

The RNA of chick embryo fibroblasts was labelled by exposing cell cultures to Eagle’s medium (8% calf’s serum) containing 1 μc/ml of 5-[3H]-uridine for 16 hr. The radioactive medium was removed and the monolayers were infected with an input m.o.i. of 100 to 300 of NDV (Texas GB strain) as described before (Huo & Wilson, 1969). These cells were incubated with Hanks’ balanced solution containing 2% calf serum. The amount of radioactive cell RNA in virus infected cells was then examined. The radioactivity in this RNA decreased with time after infection (Fig. 1 A) indicating that a portion of the RNA was degraded to acid soluble fragments. However, cell RNA was not degraded in infected cells exposed to chloroquine.

The rate of cell protein synthesis in chloroquine treated cells was also examined. At different times after infection the incorporation of [14C]-leucine into acid insoluble protein was measured (Fig. 1 B). It was observed that the rate of protein synthesis in virus-infected cells began to decrease at about 5 hr after infection and was 90% inhibited by 14 hr after infection. In infected cells exposed to chloroquine, however, the rate of protein synthesis remained near normal levels.

It has been reported that chloroquine may inhibit the growth of NDV and other viruses when the culture medium lacked serum (Inglot, 1969). The possibility that chloroquine was inhibiting NDV growth in my experiments was examined by measuring virus growth with Hanks’s balanced salt solution containing 2% calf serum as the culture medium. Under these conditions, when the medium after infection contained chloroquine diphosphate at a concentration of 50 μg./ml., the growth of NDV was normal (Table 1).

The condition of the cellular lysosomes was examined by measuring the amounts of lysosomal enzymes which were released into the overlay medium. Although very little lysosomal acid phosphatase (3.1.3.2) appeared in the medium overlying control cultures, a large amount of acid phosphatase was found in the overlay medium of infected cells at 10 hr post-infection (Table 2). There was a corresponding decrease in the acid phosphatase inside infected cells. This release of acid phosphatase is an indication of lysosomal breakdown. Chloroquine did not prevent the virus-induced release of acid phosphatase (Table 2) and therefore did not stabilize the lysosomes of infected cells. These conclusions were supported by parallel experiments in which lysosomal beta-glucuronidase (3.2.1.31) and ribonuclease
Short communications

(2.7.7.16) were examined. A similar result was obtained by Fedorko et al. (1968) who showed that chloroquine caused lysosomal breakdown and acid phosphatase release in mouse macrophages. Thus although chloroquine does protect virus infected cells, it must act in some, as yet unknown way rather than as a lysosomal stabilizer.

Fig. 1. (A) Degradation of cell RNA. The RNA in chick embryo fibroblast cultures was pre-labelled as described in the text. At different times after infection cultures were washed twice with 5% TCA and suspended in 0.3 N-NaOH for radioactivity analysis: (■) virus infected cells, (×) virus infected cells with chloroquine, (○) control cells, (○) control cells with chloroquine. (B) Cell protein synthesis. The rate of protein synthesis was measured by exposing cell cultures to medium containing 0.02 μc/ml of [3H]-leucine for 30 min. at different times after infection. Radioactivity analysis was as in Fig. 1A. The rate of protein synthesis in virus infected cells is expressed as a percentage of the rate in control cells: (■) with chloroquine in both media, or (○) without chloroquine in either.

Table 1. Growth of NDV in the presence of chloroquine

<table>
<thead>
<tr>
<th>Medium</th>
<th>p.f.u. released at different times after infection</th>
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<tbody>
<tr>
<td></td>
<td>2.5 hr</td>
</tr>
<tr>
<td>No drug</td>
<td>2.5 × 10⁴</td>
</tr>
<tr>
<td>+ 50 μg/ml. chloroquine</td>
<td>2.8 × 10⁴</td>
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Ten million cells were infected with 6 × 10⁶ p.f.u. of NDV, of which 2.8 × 10⁶ p.f.u. were actually adsorbed to the cells.
The results presented here indicated that virus replication may occur in the presence of chloroquine without degradation of cell RNA and inhibition of cell protein synthesis. Thus virus-induced injury to these cells appears to be a consequence of virus growth but not in itself a necessary event in the growth cycle of the virus.

Table 2. Acid phosphatase in chick embryo fibroblasts

<table>
<thead>
<tr>
<th>Acid phosphatase (µg.)</th>
<th>In cells</th>
<th>In medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control cells</td>
<td>411</td>
<td>3</td>
</tr>
<tr>
<td>Cells + 50 µg./ml. chloroquine</td>
<td>405</td>
<td>48</td>
</tr>
<tr>
<td>Virus infected cells</td>
<td>262</td>
<td>144</td>
</tr>
<tr>
<td>Virus infected cells + 50 µg./ml. chloroquine</td>
<td>281</td>
<td>181</td>
</tr>
</tbody>
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Cells were infected and exposed to chloroquine as described in the text. At 10 hr after infection the overlay medium was removed and the cells were lysed with 0.1% Triton X-100 in saline. Acid phosphatase was determined as described by Flanagan (1966) using crude wheat germ acid phosphatase (Worthington) as a standard. The values shown are the yield from $5 \times 10^7$ cells.

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REFERENCES


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