Effect of Nitric Oxide on Poliovirus Infection of Two Human Cell Lines

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The role of nitric oxide after poliovirus infection of the human HeLa (carcinoma) and U937 (promonocytic) cell lines has been analyzed. Both types of cells produced detectable levels of nitric oxide after poliovirus infection. However, this production was not sufficient to limit viral productivity. On the other hand, pretreatment with the nitric oxide donor glycerine trinitrate lengthened the course of poliovirus infection.

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findings (12), exogenous NO supplied by 12 h of pretreatment with 4 mg of GTN/ml produced an increase of cell viability and a 3.9- or 15-fold decrease in the PFU produced in HeLa and U937 cells, respectively, analyzed by means of a plaque assay performed on HeLa cell monolayers. This reduction of infectious particles was not due to a direct inhibitory effect of GTN on poliovirus input, since pretreatment of \(5 \times 10^6\) poliovirus particles with \(16\) mg of GTN/ml for 5 h did not alter the subsequent infectivity of the virus (data not shown). Altogether, these results indicate that the addition of NO decreases poliovirus infection in both the HeLa and U937 human cell lines. However, the low level of endogenous NO production induced after the infection does not seem to be sufficient to alter the course of poliovirus infection. Morphological studies confirmed these results (data not shown).

We further studied the effect exerted by NO on the course of protein synthesis during poliovirus infection by polyacrylamide gel electrophoretic assay. In vivo labeling of newly synthesized proteins was carried out by giving 1-h pulses with 20 \(\mu\)Ci of L-(\(35\)S)Pro-mix (approximately 70% L-[\(35\)S]methionine [\(>1,000\) Ci/mmol] and 30% L-[\(35\)S]cysteine; Amersham Life Science, Little Chalfont, Buckinghamshire, United Kingdom)/ml in methionine- and cysteine-free medium. At 4 h postinfection (p.i.), some viral proteins could be detected in infected HeLa cells (MOI, 5). The inhibition of the cellular protein was more evident at later times after infection. This shutoff was almost total at 10 h p.i. (Fig. 2). Preincubation with 4 mg of GTN/ml delayed the induction of this shutoff. Cellular protein synthesis was detected even at 10 h p.i. Cells incubated for longer times after infection underwent total cellular destruction in all cultures (data not shown). In the case of the U937 cell line.

### Table 1. Pretreatment of HeLa and U937 cells with GTN protects from poliovirus infection

<table>
<thead>
<tr>
<th>Cells</th>
<th>h.p.i.</th>
<th>Virus production&lt;sup&gt;b&lt;/sup&gt; (PFU/cell)</th>
<th>Cell viability&lt;sup&gt;c&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>24</td>
<td>63.6 ± 13.8</td>
<td>&lt;5</td>
</tr>
<tr>
<td>HeLa + l-NMMA</td>
<td>24</td>
<td>38.7 ± 8.4</td>
<td>&lt;5</td>
</tr>
<tr>
<td>HeLa + GTN</td>
<td>24</td>
<td>16.2 ± 2.3*</td>
<td>28</td>
</tr>
<tr>
<td>U937</td>
<td>54</td>
<td>24.0 ± 3.9</td>
<td>9</td>
</tr>
<tr>
<td>U937 + l-NMMA</td>
<td>54</td>
<td>28.4 ± 3.2</td>
<td>11</td>
</tr>
<tr>
<td>U937 + GTN</td>
<td>54</td>
<td>1.6 ± 0.3*</td>
<td>46</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cells were left untreated or preincubated for 4 or 12 h with 2 mM l-NMMA or 4 mg of GTN/ml, respectively. Subsequently, all cells were infected at an MOI of 1 PFU/cell. After 1 h of viral adsorption, cells were washed to remove nonadsorbed viral particles (zero time) and incubated at 37°C for the indicated times.

<sup>b</sup> The production of infectious virus was measured by plaque assay. *, significantly different from values for untreated cells (\(P < 0.001\)).

<sup>c</sup> Survival of infected cells was determined by the trypan blue exclusion technique and is expressed relative to survival of mock-infected cultures. Values are means from three independent experiments.
previous work from our laboratory demonstrated a weaker effect of poliovirus, and a longer time of infection was necessary to achieve cellular destruction (18). Moreover, this cellular death was not followed by detectable levels of viral protein synthesis. Figure 2 confirms this weaker induction of shutoff after infection for U937 cells. However, even under these conditions, preincubation with GTN protected the cells. Altogether, these results show the protection afforded by NO against poliovirus infection.

These results represent the first indication of poliovirus-mediated NO production. Nevertheless, the level of NO detected did not seem to be sufficient to ameliorate the cytotoxic effect produced by the virus. Activation of iNOS after picornavirus infection has been described only for murine systems, and the role of this endogenously produced NO remains unclear (8, 19, 21, 23). Thus, murine L-929 cells produced NO after encephalomyocarditis virus infection without counteracting viral replication (8). Another picornavirus, the cardiovirus of Theiler’s murine encephalomyelitis, is an important model of virus-induced demyelinating disease. Although infection of the susceptible SJL strain of mice with Theiler’s murine encephalomyelitis virus increased expression of iNOS, NO did not play a direct role in the late phase of demyelination (23). Furthermore, two independent groups have shown iNOS induction in the hearts of mice infected with the enterovirus CVB3 (19, 21). However, Mikami et al. (21) could not determine whether NO plays a cytotoxic or a cytoprotective role in the pathogenic mechanisms of myocardial dysfunction.

The infection of U937 cells by poliovirus described herein produced detectable levels of NO without the need of previous cellular activation. This finding contrasts with a previous study in which commitment to a more mature state of U937 cells was necessary to achieve cellular destruction (18). Moreover, this cellular activation. This finding contrasts with a previous study in which commitment to a more mature state of U937 cells was needed for the production of NO after infection by HSV-1 (16). This might suggest different pathways of iNOS induction triggered by virus infection. The role of this NO produced in vitro remains unknown. Further investigation should tackle the questions of whether infection of primary human cultures leads to the activation of iNOS and what role, if any, NO plays in an in vivo context. Regarding this point, Tucker et al. (25) suggest that NO could protect some types of cells against viral infection just until the specific immune response controls the infection.

In conclusion, it is clear from the present study that NO can delay poliovirus infection and that this picornavirus induces detectable production of NO, although probably not in sufficient amounts for the establishment of an antiviral state, at least in these culture systems.

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