Arenaviruses: Inhibition by Amantadine Hydrochloride

(Accepted 21 October 1971)

A newly defined taxonomic group, arenaviruses, has been proposed (Rowe et al. 1970a, Wildy 1971) on the basis of similarity in virus structure (Murphy et al. 1970), RNA content, sensitivity to lipid solvents, and a group specific antigen demonstrable by immunofluorescence (Rowe et al. 1970b). Lymphocytic choriomeningitis (LCM) virus was designated the prototype of the group, which presently includes Lassa fever virus and members of the Tacaribe complex of viruses. We wish to report that synthesis of all arenaviruses thus far tested is reduced in the presence of amantadine hydrochloride.

The spectrum of viruses inhibited in vitro by amantadine hydrochloride is narrow: most influenza A viruses, Sendai, pseudorabies, rubella, and Rous and Esh sarcoma (Wallbank, Matter & Klinikowski, 1966). All evidence thus far gathered with these viruses suggests that the drug inhibits only at an early stage of infection (Cochran et al. 1965; Hoffmann et al. 1965; Kato & Eggers, 1969; Maassab & Cochran, 1964). We have found, however, that this drug not only delays or prevents the penetration step in the LCM infection cycle but also inhibits a later function(s) involving virus synthesis and release (Welsh et al. 1971). Before the similarity between LCM and the Tacaribe complex of viruses was recognized the replication of one of the latter, Junin virus, was reported to be sensitive to amantadine in vitro (Coto, Calello & Parodi, 1969). We therefore determined the effect of amantadine on other members

Table 1. Inhibition by Amantadine Hydrochloride of Arenavirus Replication

<table>
<thead>
<tr>
<th>Virus (m.o.i.)</th>
<th>Cell Type</th>
<th>Drug Conc. (µg/ml.)</th>
<th>Harvest Time (hr after infection)</th>
<th>Total p.f.u. Yield in Cells†</th>
<th>Medium‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amapari (0.02)</td>
<td>Vero</td>
<td>0</td>
<td>96</td>
<td>5.5 x 10⁴</td>
<td>9.0 x 10³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>96</td>
<td>2.5 x 10⁴</td>
<td>5.0 x 10³</td>
</tr>
<tr>
<td>Parana (0.07)</td>
<td>BHK</td>
<td>0</td>
<td>48</td>
<td>6.5 x 10⁴</td>
<td>1.4 x 10³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>48</td>
<td>2.0 x 10⁴</td>
<td>3.0 x 10⁴</td>
</tr>
<tr>
<td>Tacaribe (0.005)</td>
<td>Vero</td>
<td>0</td>
<td>72</td>
<td>2.2 x 10⁴</td>
<td>3.8 x 10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>72†</td>
<td>3.3 x 10⁴</td>
<td>6.5 x 10³</td>
</tr>
<tr>
<td>Tamiami (0.001)</td>
<td>Vero</td>
<td>0</td>
<td>48†</td>
<td>2.6 x 10⁴</td>
<td>3.5 x 10³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>48</td>
<td>6.5 x 10⁴</td>
<td>1.6 x 10⁴</td>
</tr>
<tr>
<td>LCM-UBC (0.5)</td>
<td>L</td>
<td>0</td>
<td>24</td>
<td>6.0 x 10⁴</td>
<td>1.7 x 10⁷</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>24</td>
<td>3.7 x 10⁴</td>
<td>2.4 x 10⁶</td>
</tr>
</tbody>
</table>

* Vero cells required long exposure to Amantadine with the slow growing Amapari and Tacaribe viruses.
To rule out non-specific effects a control experiment was done with Dengue virus (m.o.i. 0.001). The curves for virus growth over 5 days were identical in the presence or absence of 50 µg/ml. of the drug.
† The number of cells at the time of harvest was: BHK cells – 1.5 x 10⁷; L cells – 4 x 10⁶; Vero cells – 3 to 5 x 10⁶.
‡ 5 ml. volumes.

Monolayers were prepared and infected as described previously (Welsh et al. 1971), except that the Vero cells were about 50% confluent when infected (1.5 x 10⁶ cells/small Falcon flask). The virus strains were those used by Murphy et al. (1970). Amapari and Parana viruses were obtained from K. M. Johnson, Tamiami virus from C. H. Calisher, and Tacaribe virus from ATCC. The vac strain of LCM virus was used by us previously (Pulkkinen & Pfau, 1970). The Parana virus plaque assay was that in BHK cells used for assay of LCM virus (Pulkkinen & Pfau, 1970) except that plates were stained on the 5th day after infection. Amapari, Tacaribe and Tamiami viruses were assayed on Vero cell monolayers. These plates were stained 6 days after infection.
Table 2. *Parana* virus yield with amantadine addition after complete
infective centre formation

<table>
<thead>
<tr>
<th>Hr after infection</th>
<th>Source of virus</th>
<th>Total p.f.u. with 100 µg./ml. amantadine added at 12 hr</th>
<th>Controls without amantadine</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Medium</td>
<td>9 x 10^6</td>
<td>3.8 x 10^6</td>
</tr>
<tr>
<td></td>
<td>Cells (8 x 10^5)*</td>
<td>5.5 x 10^7</td>
<td>3 x 10^7</td>
</tr>
<tr>
<td></td>
<td>Cells</td>
<td>9.5 x 10^7</td>
<td>2.9 x 10^6</td>
</tr>
</tbody>
</table>

* At 12 hr after infection the number of cells and of infective centres were identical.

Monolayers of BHK cells (6 x 10^8 cells/small Falcon flask) were exposed for 1 hr at 37° to 1 ml of *Parana* virus at 2 x 10^3 p.f.u./ml. Unadsorbed virus was decanted and the plates were washed three times with growth medium. The medium was replaced to the original 5 ml volume. Twelve hours after infection the supernatant fluid from one of the cultures was removed and assayed. The monolayer was then dispersed by trypsin, the cells washed five times and assayed for infective centres (Welsh *et al.* 1971). The cells were finally disrupted and assayed for infectivity of cell-associated virus. At the same time Amantadine (100 µg./ml.) was added to another culture and the amount of cell-associated and released virus determined in this and a control culture at 28 hr after infection.

of the arenavirus group. Duplicate sets of BHK, L, or Vero cell monolayers were infected with either Amapari, *Parana*, Tacaribe, Tamiami or LCM virus. One set of monolayers was not exposed to the drug while the other received the drug one hour before, simultaneously with, and again after infection. A third set of non-infected monolayers was exposed only to the drug to confirm absence of cytotoxicity. At various times after infection the supernatant fluids were harvested, and the monolayers dispersed either by three freeze-thaw cycles or by treatment with trypsin prior to sonic treatment. The results of these experiments (Table 1) indicate in all cases that drug concentrations of 25 to 100 µg./ml. inhibited by 40 to 95% the yields of LCM virus and of viruses of the Tacaribe complex. To determine if there was a late function of amantadine, as previously shown with LCM virus (Welsh *et al.* 1971), the drug was added to cultures infected by *Parana* virus at a time when all cells scored as infective centres. As shown in Table 2, drug addition 12 hr after infection led to significant inhibition of the yields of cell associated and released virus (67 and 58% respectively) 16 hr later. Thus, on the basis of the dual function of the drug, the type of amantadine sensitivity of the Tacaribe viruses may be similar to that of LCM virus and distinguishable from that of influenza virus. This type of drug sensitivity may be useful as a preliminary criterion in the classification of new isolates and as a tool in the analysis of the course of replication of these viruses.

Since it has been reported that amantadine modifies the syndromes in man of A2 Influenza (Togo *et al.* 1970) and Parkinson's disease (Schwab, *et al.* 1969; Völler, 1970) the possibility should be considered that this drug may be effective *in vivo* against various arenavirus infections.
This study was supported by U.S. Public Health Service Grant AI-06735 and AI-10612 from the National Institute of Allergy and Infectious Diseases and Research Career Program Award 5-K3-GM-8494 from the National Institute of General Medical Sciences.

Department of Microbiology
University of Massachusetts
Amherst, Massachusetts 01002
U.S.A.

C. J. PFAU*
R. S. TROWBRIDGE†
R. M. WELSH*
L. D. STANECK†
C. M. O’CONNELL

* Present address: Dept. of Biology, Rensselaer Polytechnic Institute, Troy, New York 12181.
† Present address: New York State Institute for Basic Research in Mental Retardation, Staten Island, N.Y. 10314.
‡ Present address: Dept. of Biology, Amherst College, Amherst, Massachusetts, 01002.

REFERENCES


(Received 26 September 1971)