In vitro culture of Toxoplasma gondii in HeLa, Vero, RBK and A549 cell lines

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SUMMARY

Toxoplasma gondii is a protozoan parasite which can be grown in vivo and in vitro. Various cell lines are used for T. gondii culture in vitro. In this study, four cell lines of HeLa, Vero, RBK and A549 were compared with each other for T. gondii tachyzoites culture. The four cell lines were cultured and infected with 5 × 10⁶ tachyzoites, respectively. The number of tachyzoites and viable host cells and pH of the media were assessed in each culture. The highest tachyzoite yield was seen in HeLa cell culture. The lowest number of viable host cells and the lowest pH were seen in HeLa cell line culture. The lowest tachyzoite yield, the highest viable cell and the highest pH were observed in Vero cell line culture. HeLa and Vero cell lines are thus appropriate for rapid and long-term propagations of T. gondii tachyzoites, respectively.

Keywords: Toxoplasma gondii, cell culture, cell line.

INTRODUCTION

Toxoplasma gondii, a protozoan parasite with world-wide distribution, is the agent of toxoplasmosis in humans [1]. In vivo and in vitro production of T. gondii tachyzoites is critical for Toxoplasma researches [2]. Due to ethical issues and infra structural deficiencies in in vivo culture models such as animal models, these models have been replaced by cell culture systems [3]. Toxoplasma gondii tachyzoites can be cultured and maintained in various culture systems and cell lines [4]. These tachyzoites are used in fundamental studies and diagnostic assays [5-9]. Furthermore, cell culture systems can be used in T. gondii cyst formation [10]. The aim of this study included comparison of the four cell lines of HeLa (human cervix carcinoma), Vero (African green monkey kidney carcinoma), RBK (Razi bovine kidney) and A549 (human lung carcinoma) for culture of T. gondii tachyzoites in vitro.

MATERIALS AND METHODS

Preparation of T. gondii tachyzoite
Tachyzoites of T. gondii (RH strain) were inoculated intraperitoneally into BALB/c mice. After 3-4 days, tachyzoites were harvested using intraperitoneal wash with phosphate buffered saline (PBS; pH 7.3) and then were counted. Adherent cell lines of HeLa, Vero, RBK and A549 were grown in 10 mL of Dulbecco’s Modified Eagle culture media (DMEM; KB cell, Iran), supplemented with 10% of inactivated fetal calf serum (FCS; Boivogen, Australia), 10 µg of hepes and 1% of penicillin-streptomycin (Biovest, France) using 25 cm² flasks (Nunc, Denmark) and then were incubated in 5% CO₂.

After formation of confluent monolayers, the media was replaced by the maintenance media (DMEM/hepes with 5% FCS).
Cell lines were infected 1:1 by tachyzoites of *T. gondii*, RH strain. Therefore, each flask was infected with 50×10⁶ tachyzoites. The culture media was replaced after 4 and 24 h by DMEM without FCS. Flasks were investigated on Days 3 and 5 post infection. Number of tachyzoites, viability of cell lines and pH of media were calculated on Days 3 and 5 post infection.

### RESULTS

Number of tachyzoites of *T. gondii*, RH strain, in the four cell lines (HeLa, Vero, RBK and A549), number of the host cells and pH of each flask were shown in Table 1. On Days 3 and 5 post infection, number of derived tachyzoites from the four cell lines were in the following order of HeLa > RBK > A549 > Vero. The highest and the lowest numbers of tachyzoites of *T. gondii*, RH strain, on Days 3 and 5 post infection were seen in HeLa and Vero cell lines, respectively. On Days 3 and 5 post infection, pH of the culture media for these four cell lines was as follows: A549 > RBK > Vero > HeLa. The pH of culture medium is an indicator of tachyzoite reproduction, so the lowest pH was seen in HeLa cell line in comparison with the others. The order of viable host cells in these four cell lines on Days 3 and 5 post infection was as follows: Vero > A549 > RBK > HeLa. The lowest number of viable cells was seen in HeLa cell line, indicating multiplication of the tachyzoites and hence rupture of the host cells in this cell line. Comparison of the tachyzoite yields in these four cell lines with tachyzoites of *T. gondii*, RH strain, on Days 3 and 5 post infection is shown in Figure 1.

### DISCUSSION

Propagation of *Toxoplasma* tachyzoites in cell culture systems includes multiple advantages compared to that in animal models. These advantages include ethical value, low cost and easy management [11]. Various cell lines have been used to produce *T. gondii* tachyzoites *in vitro* [12-14]. Unlike passage in mice, passage of tachyzoites in cell cultures is not known to alter the virulence of the microorganism. Tachyzoites multiply in almost all mammalian cell lines. Yield of tachyzoites varies based on the cell line and strain of *T. gondii*. Mean generation time of the tachyzoites of *T. gondii*, RH strain, is reported nearly 5 h (15). In the present study, four cell lines were compared with each other for *T. gondii*, RH strain, cell culture. At the same condition, HeLa cell line was shown more appropriate than Vero, RBK and A549. Low levels of the host cell contamination and high levels of tachyzoite yield were seen in HeLa cells on Days 3 and 5 post infection. In Vero cell line, the highest contamination and the lowest yield rates of tachyzoites were observed. Furthermore, pH of the culture media was more acidified in HeLa cell culture on Days 3 and 5 post infection than that of the other cell lines, indicating high reproduc-

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Yield of tachyzoites (×10⁶/ml)</th>
<th>Viable host cells (×10⁶/ml)</th>
<th>pH of media</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>60.2</td>
<td>2.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Vero</td>
<td>107.7</td>
<td>1.05</td>
<td>7.53</td>
</tr>
<tr>
<td>RBK</td>
<td>53</td>
<td>2.4</td>
<td>7.82</td>
</tr>
<tr>
<td>A549</td>
<td>90.1</td>
<td>1.91</td>
<td>7.62</td>
</tr>
</tbody>
</table>

**Figure 1** - Comparison of tachyzoite yields in HeLa, Vero, RBK and A549 cell lines on Days 3 and 5 post infection with *T. gondii*, RH strain.
tion rate of the parasite. Hughes et al. compared four cell lines of HEP2, Vero, MRC5 and AGMPK for in vitro T. gondii culture. They found that optimal growth conditions occurred in AGMRK cell line [16]. Evans et al. used three cell lines of HeLa, LLC and Vero for continuous production of T. gondii tachyzoites and investigated that HeLa cell line included a higher tachyzoite yield than that LLC and Vero cells did [17]. In the present study, RBK and A549 cell lines were ranked second and third based on the tachyzoite yield, host cell contamination and pH of media. Therefore, RBK cell line can be substituted by HeLa cell line to have a large number of tachyzoites in cell culture. The Vero cell line seems to be appropriate for the long-time production of T. gondii tachyzoites in cell cultures.

**CONCLUSION**

Of the assessed four cell lines of HeLa, Vero, RBK and A549 for T. gondii cell culture, HeLa is the best and Vero is the worst due to the number of derived tachyzoites. However, the Vero cell line is appropriate for the long-time culture of the parasite.

**ACKNOWLEDGMENT**

This study was carried out as a MSPH thesis. The study was financially supported by Tehran University of Medical Sciences and approved by the University’s Ethical Committee.

**Conflict of interest**

The authors declare no conflict of interest.

**REFERENCES**


