Microbial Growth in Various Perfluorocarbon Liquids

Lider Celik, MD
Mine Yücesoy, Prof. Dr.
A. Osman Saatci, Prof. Dr.
Süleyman Kaynak, Prof. Dr.

Abstract. A prospective in vitro study was conducted to investigate the potential for various perfluorocarbon liquids to support the growth of microbes, which may be introduced into these liquids as contaminants during intraocular surgery. Perfluorodecaline, perfluoro-n-octane, and perfluorophenanthrene were tested for the growth of Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans by using tryptone soy broth, pancreatic digest of casein, and Sabouraud broth as culture media for up to 10 days. No microbial growth was observed in any of these perfluorocarbon liquids. Perfluorocarbon liquids do not promote microbial growth. Thus, they do not increase the risk of endophthalmitis in vitreoretinal surgery. [Ophthamic Surg Lasers Imaging 2005;36:254-257.]

INTRODUCTION

Perfluorocarbon liquids have been introduced into the field of vitreoretinal surgery due to their physical and chemical properties, such as transparency, high specific gravity, low viscosity, and immiscibility with water. They provide valuable assistance as an intraoperative adjunct, and clinical indications for the perfluorocarbon liquids have grown rapidly. They are widely used as a temporary tamponade agent during surgical procedures. Surgeons often prefer to remove perfluorocarbon liquids intraoperatively as completely as possible, but residual droplets may persist. In selected cases, some surgeons may elect to use perfluorocarbon liquids as a long-term replacement.

We performed a prospective in vitro study to investigate the potential for various perfluorocarbon liquids (perfluorodecaline, perfluoro-n-octane, and perfluorophenanthrene) to support the growth of microbes, which may be introduced into these liquids as contaminants during intraocular surgery.

DESIGN AND METHODS

Perfluorocarbons

Perfluoro-n-octane (Perfluron; Alcon Laboratories, Fort Worth, TX), perfluorodecaline (DK-line; Chauvin Opsia France, Toulouse, France), and perfluorophenanthrene (Vitreon; Vitrophage Inc., Lyons, IL) were tested in this study.

Microbiologic Agents

Two bacteria (Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853) and one fungus (Candida albicans ATCC 90028) were inoculated.

Culture Media

Tryptone soy broth (International Gray Diagnostics; Lancashire, UK), pancreatic digest of casein (Acumed Manufacturers, Baltimore, MD), saline solution, and Sabouraud broth were used.

Procedure

Six groups of culture media were prepared. Each group consisted of seven sterile flasks. The flasks in group 1 contained 1 mL of perfluoro-n-octane, the flasks in group 2 contained 1 mL of perfluorodecaline, the flasks in group 3 contained 1 mL of perfluorophenanthrene, the flasks in group 4 contained 1 mL of perfluorodecaline and 1 mL of perfluorophenanthrene, the flasks in group 5 contained 1 mL of perfluorodecaline and 1 mL of perfluoro-n-octane, and the flasks in group 6 contained 1 mL of perfluorophenanthrene and 1 mL of perfluoro-n-octane.
TABLE 1

<table>
<thead>
<tr>
<th>Culture Time</th>
<th>Perfluoro-octane</th>
<th>Perfluorodecaline</th>
<th>Perfluorophenanthrene</th>
<th>Tryptone Soy Broth</th>
<th>Pancreatic Digest of Casein</th>
<th>Saline Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time zero</td>
<td>4-50 × 10^2</td>
<td>3 × 10^2</td>
<td>1-50 × 10^2</td>
<td>10^7</td>
<td>10^7</td>
<td>10^7</td>
</tr>
<tr>
<td>72 hours</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>10^7</td>
<td>10^7</td>
<td>2-6 × 10^3</td>
</tr>
<tr>
<td>168 hours</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>10^7</td>
<td>10^7</td>
<td>2-10 × 10^2</td>
</tr>
<tr>
<td>240 hours</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>10^7</td>
<td>10^7</td>
<td>10^4</td>
</tr>
</tbody>
</table>

CFU = colony-forming units.

TABLE 2

<table>
<thead>
<tr>
<th>Culture Time</th>
<th>Perfluoro-octane</th>
<th>Perfluorodecaline</th>
<th>Perfluorophenanthrene</th>
<th>Tryptone Soy Broth</th>
<th>Pancreatic Digest of Casein</th>
<th>Saline Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time zero</td>
<td>10^5</td>
<td>1-70 × 10^2</td>
<td>14-29 × 10^2</td>
<td>10^7</td>
<td>10^7</td>
<td>10^7</td>
</tr>
<tr>
<td>72 hours</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>10^7</td>
<td>10^7</td>
<td>10^7</td>
</tr>
<tr>
<td>168 hours</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>10^7</td>
<td>10^7</td>
<td>10^7</td>
</tr>
<tr>
<td>240 hours</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>10^7</td>
<td>10^7</td>
<td>10^7</td>
</tr>
</tbody>
</table>

CFU = colony-forming units.

anthrene, the flasks in group 4 contained 1 mL of tryptone soy broth, the flasks in group 5 contained 1 mL of pancreatic digest of casein, and the flasks in group 6 contained 1 mL of saline solution. The first and second flasks of each group were inoculated with one full colony of a 24-hour culture of S. aureus. Similarly, the third and fourth flasks of each group were inoculated with P. aeruginosa and the fifth and sixth flasks with C. albicans. Flask 7 of each group served as a control without contamination. To observe the bioavailability of C. albicans colonies, this microorganism was also inoculated into Sabouraud broth.

After the inoculation step, all flasks were homogenized by using a vortex (1,000 rpm) and incubated at 37°C for 10 days. Subcultures to blood agar plates were prepared at time zero (immediately after the vortex homogenization step), and after 72, 168, and 240 hours by using both a calibrated loop (0.01 mL) and a micropipette (0.01 mL). Both methods were applied to ensure the adequacy of materials replated. The blood agar plates were incubated at 37°C for 24 hours and the growth of microorganisms was determined by colony counting. The results were given as colony-forming units (CFU)/mL.

**FINDINGS**

No growth of any microorganism was detected in the control flasks of each group of media for all time periods. Growth was detected only at time zero subcultures of the three perfluorocarbon liquids. However, subcultures from the perfluorocarbon liquids at 72, 168, and 240 hours revealed no further survival of any type of microorganism. (Tables 1-3). We observed growth of 10^5 to 10^7 CFU/mL of all three microorganisms in tryptone soy broth and pancreatic digest of casein at all times beginning with time zero subculture. At time zero subculture from saline solution, all three microorganisms showed a proliferation of 10^7 CFU/mL, with decreasing colony counts of S. aureus but persistent ubiquitous proliferation of P. aeruginosa at subsequent replating periods. Growth of C. albicans in saline solution was 10^4 CFU/mL at 10 days. C. albicans also showed proliferation in Sabouraud broth, as expected, with 10^7 CFU/mL at time zero and decreasing to 10^3 to 10^4 CFU/mL.
**TABLE 3**

**Colony Counts of Candida albicans at the Subcultures of Perfluorocarbon Liquids and Different Culture Media (CFU/mL)**

<table>
<thead>
<tr>
<th>Culture Time</th>
<th>Perfluoro-octane</th>
<th>Perfluorodecane</th>
<th>Perfluorophenanthrene</th>
<th>Tryptone Soy Broth</th>
<th>Pancreatic Digest of Casein</th>
<th>Saline Solution</th>
<th>Sabouraud Broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time zero</td>
<td>No growth</td>
<td>No growth</td>
<td>2-70 × 10^2</td>
<td>10^6</td>
<td>10^6</td>
<td>10^5</td>
<td>10^7</td>
</tr>
<tr>
<td>72 hours</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>10^7</td>
<td>10^7</td>
<td>10^5 to 10^6</td>
<td>10^7</td>
</tr>
<tr>
<td>168 hours</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>10^7</td>
<td>10^6 to 10^7</td>
<td>10^6 to 10^7</td>
<td>10^6 to 10^7</td>
</tr>
<tr>
<td>240 hours</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>10^7</td>
<td>10^6 to 10^7</td>
<td>10^5 to 10^5</td>
<td>8-100 × 10^3</td>
</tr>
</tbody>
</table>

*CFU = colony-forming units.*

**DISCUSSION**

Endophthalmitis after pars plana vitrectomy is rare. Kattan et al.\(^7\) reported the incidence of endophthalmitis as 1 in 1,974 pars plana vitrectomies (0.05%). However, visual prognosis is poor in patients who developed endophthalmitis after vitrectomy procedures. There are several reasons for this grim visual outcome.\(^1\) Patients undergoing vitrectomy already have complicated posterior segment changes and may have very poor visual potential. External prophylaxis may have little effect on microorganisms that gain direct access to the vitreous cavity. Moreover, sterile inflammation may occur after vitrectomy due to lengthy and complicated surgeries. Therefore, the best idea for prevention is to eliminate all risk factors contributing to endophthalmitis in eyes undergoing vitrectomy.

May intentional or unintentional residual perfluorocarbon liquids support the growth of microbes? Moreira et al.\(^12\) recently performed the only study evaluating the growth of *S. aureus* and *P. aeruginosa* in perfluoro-octane and found that perfluoro-octane was not a favorable medium for bacterial growth and also was not prone to bacterial contamination during vitreo-retinal surgery. In our study, *P. aeruginosa* and *S. aureus* did not grow in any of the perfluorocarbon liquids except at time zero, which corresponded to the time of inoculation. *C. albicans* did not grow in perfluoro-octane and perfluorodecaline even at time zero. In addition to the perfluoro-octane used by Moreira et al., we also tested perfluorodecaline and perfluorophenanthrene. Lack of nutrients alone would not be sufficient to explain the absence of growth of these agents because they grew abundantly in saline. Also, there was no microbial growth in perfluorocarbon liquid samples used as uncontaminated controls, and this was proof that no external contamination occurred during manipulation of the perfluorocarbon liquids.

In light of our study, we believe that all three of the most widely used perfluorocarbon liquids (perfluorodecaline, perfluoro-octane, and perfluorophenanthrene) carry no risk of microbial contamination and do not promote microbial growth in vitreo-retinal surgeries.

**REFERENCES**


