Tissue Culture Study of Adult Human Retina Neurons

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Explant cultures were established from adult human retina tissues obtained from 20 individuals (age range 19–79), 7–45 hr postmortem, and maintained for the period of up to 4 months. Forty percent of these cultures (eight out of 20 cases) produced healthy and viable cultures as judged by phase contrast microscopy and by electron microscopy. Phase contrast microscopic examination of living cultures showed that the early outgrowths of the explants consisted of flat fibroblasts migrating out from the edge of the explants. For the next 2–4 weeks, a large population of small spherical or ovoid cells possessing thin processes was found in the areas of the outgrowth. Electron microscopic examination of cultures revealed the survival of photoreceptors, neurons and synapses with well preserved ultrastructures. This communication is the first to describe the successful culture of adult human CNS neurons in general and adult human retina neurons in particular. This culture system is ideally suited to investigate the effects of infective or toxic agents suspected of causing retinal pathology in human eye diseases. Invest Ophthalmol Vis Sci 29:1372–1379, 1988

Previously, tissue culture studies of neural retina used fetal, embryonic or neonatal animals as sources of tissues.1–6 Tissue culture of adult avian or mammalian retina had rarely been performed in the past, the only exceptions being our previous study reporting the culture of adult dog retina explants7 and the culture of dispersed adult human retina.8 In the former, electron microscopical examination of retina explants demonstrated the survival of neurons with intact synaptic connections, photoreceptors and glial cells. Bodian silver staining revealed the continued presence of mature neurons with extensive regeneration of axonal processes.7 In the latter, adult human retina was dispersed by trypsin and grown on feeder layers. Some of surviving cells were identified as neurons by tetanus toxin immunostaining and by the incorporation of neurotransmitters.8

In the current report, we describe the successful explant culture of adult human retina tissues obtained 7–45 hr postmortem, and provide electron microscopic evidence for the survival of retinal neurons and preservation of their ultrastructures.

Materials and Methods

Retina tissues were isolated from eyes of 20 donors as shown in Table 1. The eyes were received from the Eye Bank of British Columbia and the donor consents were duly obtained. The time elapsed between the donor's time of death and the isolation of retina for the culture ranged from 7 to 45 hr. The globes were stored in humidified bottles at 4°C until processing as described below. Following the removal of corneal tissues, remaining globes were transported from the Eye Bank to our laboratory, which is located 3 miles away.

Following coronal section of the globe at the equatorial plane, the vitreous was removed, the retina was peeled off and placed in Hanks' balanced salt solution (BSS). The retina was sliced into 2 × 2 mm explants with two scalpels. Each retina explant was placed on collagen-coated round Aclar plastic coverslips (12 mm diameter) and covered with a drop of feeding medium. The feeding medium consisted of 10% fetal calf serum, 5 mg/ml glucose, 20 μg/ml gentamicin and 5 μg/ml fungizone in Eagle's minimum essential medium (Gibco, Grand Island, NY). The medium was then introduced slowly and gently over the explants. The feeding medium consisted of 10% fetal calf serum, 5 mg/ml glucose, 20 μg/ml gentamicin and 5 μg/ml fungizone in Eagle's minimum essential medium (Gibco, Grand Island, NY). The medium...
Table 1. Adult human retina explant cultures

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Cause of death</th>
<th>Enucleation after death (hours)</th>
<th>Culture after enucl. (hours)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>374</td>
<td>29</td>
<td>M</td>
<td>Head injury</td>
<td>5</td>
<td>19</td>
<td>Excellent</td>
</tr>
<tr>
<td>392</td>
<td>55</td>
<td>M</td>
<td>Carcinoma (lung)</td>
<td>6</td>
<td>24</td>
<td>Excellent</td>
</tr>
<tr>
<td>396</td>
<td>74</td>
<td>M</td>
<td>Cardiac arrest</td>
<td>3</td>
<td>21</td>
<td>Poor</td>
</tr>
<tr>
<td>412</td>
<td>32</td>
<td>M</td>
<td>Asphyxia</td>
<td>5</td>
<td>19</td>
<td>Excellent</td>
</tr>
<tr>
<td>423</td>
<td>31</td>
<td>M</td>
<td>Overdose</td>
<td>3</td>
<td>19</td>
<td>Good</td>
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<tr>
<td>440</td>
<td>26</td>
<td>M</td>
<td>Cerebral aneurysm</td>
<td>20</td>
<td>10</td>
<td>Poor</td>
</tr>
<tr>
<td>448</td>
<td>62</td>
<td>F</td>
<td>Cerebral hemorrhage</td>
<td>4</td>
<td>10</td>
<td>Poor</td>
</tr>
<tr>
<td>449</td>
<td>35</td>
<td>M</td>
<td>Gunshot (head)</td>
<td>3</td>
<td>27</td>
<td>Excellent</td>
</tr>
<tr>
<td>459</td>
<td>19</td>
<td>M</td>
<td>Chest injury</td>
<td>5</td>
<td>15</td>
<td>Fair</td>
</tr>
<tr>
<td>463</td>
<td>79</td>
<td>M</td>
<td>Carcinoma (colon)</td>
<td>28</td>
<td>17</td>
<td>Poor</td>
</tr>
<tr>
<td>471</td>
<td>70</td>
<td>M</td>
<td>Emphysema</td>
<td>4</td>
<td>26</td>
<td>Fair</td>
</tr>
<tr>
<td>473</td>
<td>28</td>
<td>F</td>
<td>Astrocytoma</td>
<td>4</td>
<td>9</td>
<td>Excellent</td>
</tr>
<tr>
<td>477</td>
<td>63</td>
<td>M</td>
<td>Cardiac arrest</td>
<td>4</td>
<td>24</td>
<td>Fair</td>
</tr>
<tr>
<td>490</td>
<td>67</td>
<td>M</td>
<td>Carcinoma (lung)</td>
<td>8</td>
<td>15</td>
<td>Poor</td>
</tr>
<tr>
<td>492</td>
<td>58</td>
<td>M</td>
<td>Cardiac failure</td>
<td>2</td>
<td>30</td>
<td>Fair</td>
</tr>
<tr>
<td>518</td>
<td>53</td>
<td>M</td>
<td>Burn</td>
<td>7</td>
<td>17</td>
<td>Good</td>
</tr>
<tr>
<td>521</td>
<td>61</td>
<td>M</td>
<td>Respiratory failure</td>
<td>5</td>
<td>8</td>
<td>Excellent</td>
</tr>
<tr>
<td>530</td>
<td>20</td>
<td>M</td>
<td>Head injury</td>
<td>1</td>
<td>12</td>
<td>Good</td>
</tr>
<tr>
<td>531</td>
<td>37</td>
<td>F</td>
<td>Cerebral hemorrhage</td>
<td>1</td>
<td>12</td>
<td>Excellent</td>
</tr>
<tr>
<td>537</td>
<td>24</td>
<td>M</td>
<td>Head injury</td>
<td>3</td>
<td>4</td>
<td>Excellent</td>
</tr>
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</table>

was renewed once a week. At various times, cultures were examined under a Nikon inverted microscope and photographs were taken with Kodak Plus-X film at ASA 100.

For electron microscopy, Aclar plastic coverslips carrying retina explants were fixed in 3% glutaraldehyde in 0.12 M phosphate buffer (pH 7.4) for 30 min at room temperature, followed by fixation in 2% osmium tetroxide in the same buffer for another 30 min, dehydrated and embedded in EMBed 812 (EM Sciences, Fort Washington, PA). Semithin sections were prepared from the blocks, stained with 1% toluidine blue, and specific areas were selected for ultrathin sectioning. Ultrathin sections were stained by lead citrate and uranyl acetate, and examined under a Philips 300 electron microscope.

Results

A considerable variation between experiments in the number of explants surviving in culture was found in 20 series of cultures (Table 1). The results indicate that the time elapsed between the donor's time of death and enucleation was the most critical factor for the survival of explants. While retina tissues from globes enucleated 1-6 hr postmortem survived and grew well in culture, the globes isolated 8-28 hr postmortem did not yield any positive result. It is also evident that the time between the enucleation of the eye and the actual set-up of cultures is not crucial, as evidenced by the survival of explants obtained from a retina tissue isolated 30 hr postmortem (24 hr between enucleation and culture set-up).

The first sign of outgrowth from the explant usually was noted by 5-7 days in vitro and consisted of a few elements of flat cells easing out from the edge of the explant (Fig. 1). For the next 2-4 weeks, outgrowths of flat cells, probably fibroblasts, continued and expanded to form a broad ring surrounding the explant. Following this, a large number of small spherical or ovoid cells (7-10 μm in diameter), possessing thin processes, migrated out of the explants and located themselves on the top of the flat cell layer (Fig. 2). The overall gross appearance of cultures did not change or alter for a period as long as 4 months in vitro.

The human retina contains the cellular elements of rod and cone photoreceptors, bipolar neurons, horizontal cells, amacrine cells, ganglion cells and Müller glia cells, and these cells are organized into a well defined layered cytoarchitecture. During the entire period of culture, often of up to 4 months, most of the explants retained a considerable degree of histotypic organization, with clearly defined layers of photoreceptor, inner nuclear, inner plexiform and ganglion cell layers. Such well preserved retinal cytoarchitecture is evident in semithin sections prepared from a retina explant culture grown for 28 days (Fig. 3). Similarly, at the ultrastructural level, the layered arrangement of cell types enabled us to identify photoreceptors, interneurons of bipolar and horizontal cells, ganglion cells and possibly Müller cells (Fig. 4).
The photoreceptor cell layer showed densely packed cells with dark, round nuclei, condensed chromatin and cytoplasm with full components of mitochondria, Golgi apparatus and endoplasmic reticulum. Zona adherens were prominent structures connecting neighboring photoreceptors. Well differentiated outer segments were not observed, but somewhat disorganized outer segments were frequently encountered (Fig. 5).

In the inner nuclear layer, many small neurons were demonstrated (Fig. 6). These cells were separated by processes of Müller cells and possessed spherical nuclei with a dense chromatin network and cytoplasm containing scanty organelles.

A considerable variation in the numbers of surviving neurons between explants was noted, particularly in small neurons of the inner nuclear layer. Examination of semithin cross-sections of the explants showed...
that approximately 40–200 neurons per section (average 2 × 0.4 mm in size) survived in culture after 4–17 weeks.

The number of ganglion cells surviving was far less than that of small neurons and usually numbered one to several within a whole section (the number can be higher in whole explants). In the inner plexiform layer, a considerable number of synapses were observed. These included ribbon synapses and other conventional axodendritic synapses (Fig. 7). The most interesting aspect of the synaptic structures was the observation of reciprocal synapsis (Fig. 8). This type of synapse is typically found in vertebrate retina and is characterized by the presence of two adjacent synaptic junctions between two neuronal processes. The presence of the reciprocal synapsis in the culture indicates the survival of amacrine cells and bipolar cells in retina explants, since this type of synapse is formed by these two local circuit neurons of retina.12

Müller cells, which are glial cells unique to retina, were also found in every layer of the explants, and were readily identified ultrastructurally by the presence of 10 nm intermediate filament bundles in their cytoplasm (Fig. 9).

**Discussion**

We demonstrated in the current report that retinal neurons of adult human obtained within 30 hr of death can be maintained in culture for more than 4 months. Electron microscopic observation of both presynaptic and postsynaptic structures in the neuropil provided unequivocal evidence that a large number of neurons survived and regenerated in tissue culture.

Several groups have previously cultured adult mammalian peripheral nervous system neurons in vitro,13–16 but reports of successful culture of adult central nervous system (CNS) neurons have been much less convincing.17–20 These previous reports claimed that healthy adult CNS neurons were present in the cultures, but a careful examination of illustrations accompanying these claims, which are mostly of phase contrast micrographs of process-bearing cells, suggests that these authors mistakenly identified fibroblasts or glial cells as nerve cells. None of these authors applied electron microscopy, immunochemical or electrophysiological techniques to verify their neuronal identification. Many of these previous attempts at culturing CNS neurons used cerebral or cerebellar cortical tissues. The retina is a more suitable material for explant culture than cerebral or cerebellar cortex. This is because the retina is composed as a thin sheet such that the dissection and trimming of retina into smaller explants involves minimal disruption of structural integrity. In addition, an increased rate of diffusion of oxygen and nutrients into the thin retina tissue may improve the survival of neural elements. These factors may give retina neurons time to adapt to the culture environment and to survive in culture for an extended period of time as compared to other regions of the CNS.

Previously, Oka et al have reported the long-term culture of adult human retina cells dispersed by trypsin and grown on astrocyte feeder layers.8 These authors were able to identify neurons by positive immunostaining by tetanus toxin antibody and the incorporation of tritiated neurotransmitter candidates, such as γ-aminobutyric acid, glycine and dopamine.8 The current study confirms their results: adult
Fig. 4. This low-power electron micrograph represents a cross-section of the full thickness of a retina explant. A 35-day-old culture of a retina explant obtained from 35-year-old male 30 hr postmortem. Bar = 5 μm. OLM: outer limiting membrane, ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GL: ganglion cell layer, ILM: inner limiting membrane.
Fig. 5. This electron micrograph shows outer limiting membrane (OL), rod inner segments (RI), a disorganized rod outer segment (RO) and a rod cell nucleus. Retina from 35-year-old male 30 hr postmortem and grown for 21 days in vitro. Bar = 1 μm.

Fig. 6. Several neurons in the outer zone of the inner nuclear layer are shown here. They are mostly of bipolar cell neurons. Retina from 29-year-old male 24 hr postmortem and grown for 68 days in vitro. Bar = 1 μm.

human retina neurons can survive in culture if culture conditions are optimal. Human retina culture as described in this report can serve as a useful model system in which to study the basic morphological and physiological characteristics of human retina neurons, to determine the re-
Fig. 7. Several synaptic profiles found in the outer plexiform layer are shown here. Bar = 1 μm. Two of these synapses containing synaptic ribbons are shown in insets at the higher magnification. Bar in the inset = 0.5 μm. Fig. 8. Two synaptic profiles are found in the inner zone of the outer plexiform layer. One of them represents a reciprocal synapse (RS) in which two synaptic contacts of opposing polarity are located in a same terminal. Retina from 29-year-old male 24 hr postmortem grown for 68 days. Bar = 1 μm.

Generative ability of these neurons, and to study the structural changes of these neurons with advanced age. In addition, this culture system is ideally suited to investigate the effects of infective or toxic agents suspected of causing retinal pathology in human eye diseases.
Fig. 9. This electron micrograph shows a Müller cell located in the inner nuclear layer. Several profiles of Müller cell processes containing bundles of intermediate filaments are also shown here. Retina from 26-year-old male 30 hr postmortem grown for 35 days in vitro. Bar = 1 μm.

Key words: electron microscopy, human, neurons, retina, synapses, tissue culture

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References