Measurement of Intraocular Pressure by Both Invasive and Noninvasive Techniques in Rabbits Exposed to Head-Down Tilt

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Abstract: This study investigates changes in intraocular pressure (IOP) in rabbits during head-down tilt (HDT), which is commonly used as an experimental model to simulate microgravity. IOP was measured by the needle insertion technique (IOP\textsubscript{NEEDLE}) and Tono-pen tonometry (IOP\textsubscript{TONO-PEN}). Although the absolute value of the IOP\textsubscript{TONO-PEN} was significantly smaller than that of the IOP\textsubscript{NEEDLE}, a significant correlation ($r=0.99$) was observed between them. A linear regression analysis yielded an equation as follows: $\text{IOP}_{\text{TONO-PEN}} = 0.67 \times \text{IOP}_{\text{NEEDLE}} - 0.67$. Both the IOP\textsubscript{NEEDLE} and the IOP\textsubscript{TONO-PEN} changed depending on the tilt angle. Tilting from horizontal ($0^\circ$) to 75° head-down increased the IOP\textsubscript{NEEDLE} and the IOP\textsubscript{TONO-PEN} by $7.3\pm 0.8$ (mean±SEM) mmHg and $4.4\pm 1.3$ mmHg. The IOP\textsubscript{NEEDLE} elevated from $13.1\pm 1.3$ to $16.9\pm 1.0$ mmHg immediately after the onset of 45° HDT and then gradually declined. The value of the IOP\textsubscript{NEEDLE} during 8 h of HDT was significantly higher than the value in the control animals, which were kept at the horizontal prone position throughout the experiment. Similar findings were observed in the IOP\textsubscript{TONO-PEN}. These results suggest that the needle insertion technique and the Tono-pen tonometry are both useful for measuring IOP in rabbits. [Japanese Journal of Physiology, 48, 25–31, 1998]

Key words: microgravity, head-down tilt, intraocular pressure, Tono-pen tonometry, needle insertion technique.

Exposure to actual or simulated microgravity causes cephalad fluid shift, which changes circulation in the head [1]. A recent study indicated that HDT at 6°, which is a widely accepted experimental model for microgravity [2, 3], increased capillary pressure in the head and decreased plasma colloid osmotic pressure [1]. These hemodynamic changes may explain nasal congestion and facial edema commonly observed in astronauts during space flight [4] or in subjects exposed to simulated microgravity [1, 5].

Furthermore, several studies demonstrated that intraocular pressure (IOP) elevates during space flight [6], parabolic flight [7], and HDT [8–12]. Linder and colleagues [10, 11] suggested that HDT produced a significant reduction in the neurophysiological function as measured by pattern-reversal electroretinogram and visually evoked potential in human subjects.

In the previous studies, changes in IOP were measured intermittently in human subjects by using noninvasive techniques. To our knowledge, there are no reports in which the changes of IOP during HDT were continuously investigated in animal experiments, except for our previous report [13]. Draeger and co-workers [6] demonstrated that during a space mission, IOP in an astronaut increased 92% above the baseline value 16 min after the onset of microgravity, but it returned to the baseline after 6 h and 18 min. To examine the precise time course of IOP during exposure to microgravity, continuous measurement by means of a direct, i.e., invasive, technique will be necessary. Since such technique cannot be available in human studies, it is of worth to develop a model in an animal experiment.

The purpose of this study is to compare the changes
in IOP measured by the Tono-pen tonometry technique (noninvasive) with those measured by the needle insertion technique (invasive). We therefore measured IOP before, during, and after HDT in rabbits by using the invasive and noninvasive techniques. Then we investigated what degree of HDT is most appropriate as an experimental model to simulate microgravity in rabbits. The time course of IOP during HDT of 8 h was also examined by means of the invasive and noninvasive techniques. Our data could give useful information in the fields of IOP, ocular hemodynamics, and visual functions during the early stage of microgravity.

**METHODS**

Twenty-four adult male and female albino rabbits weighing 2.3–3.2 kg, were used in this study. All procedures were reviewed and approved by the Committee for Animal Experimentation in the Faculty of Medicine, Tottori University, Japan, and conformed to Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences published by the Council of the Physiological Society of Japan.

**Animal preparation.** The rabbits were anesthetized with an intravenous injection of 25 mg/kg pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, Ill.). An additional dose (1 mg/kg/h) of pentobarbital sodium was administered by using a syringe pump (STC-521, Terumo, Tokyo) through a polyethylene catheter (4 Fr) inserted into the right femoral vein [14]. Each rabbit was paralyzed with pancuronium (Mioblock, Organon, Oss, The Netherlands)—an initial dose of 0.2 mg/kg and additional doses as necessary, i.m.—and artificially ventilated (60 cycle/min, 5 ml/kg) with room air by a mechanical ventilator (SN-480-6, Shinano, Tokyo) to maintain $P_{\text{ac}}$ at 30–40 mmHg. The right femoral artery was cannulated with a polyethylene catheter filled with physiological salt solution (Otsuka, Tokyo) containing 101U/ml of heparin (Heparin Sodium Injection-N, Takeda, Osaka) for the measurement of arterial pressure and blood gas analysis (model 1306, Instrumentation Laboratory, Milano, Italy). For the measurement of $P_{\text{ac}}$, 0.3 ml of arterial blood was drawn through a three-way stopcock. The same amount of physiological salt solution was injected to replace the blood. The arterial pressure was measured by a pressure transducer (TP-400T, Nihon Kohden, Tokyo) which was fixed at the same height as the tip of the arterial catheter and recorded on a thermal array recorder (RTA-4100, Nihon Kohden). Rectal temperature was maintained from 38 to 40°C by using a heating pad. After these procedures were completed, the rabbits were mounted in a stereotaxic frame that could be rotated in pitch.

**Measurements of IOP.** A 25-gauge needle was inserted into the anterior chamber of the left eye and connected with a pressure transducer (TP-400T, Nihon Kohden) which was secured at the same height as the tip of the needle. The output of the transducer was recorded on a thermal array recorder (RTA-4100, Nihon Kohden). IOP was also measured noninvasively by means of the Tono-pen tonometry [15] in the same eye. The stainless steel probe on Tono-pen (TONO-PEN XL, Mentor, Norwell, USA) contained a solid state strain gauge that converted IOP to an electrical signal. A microprocessor and electronics housed in the body of the instrument were used to analyze the signal produced by each touch to the cornea surface, and each single valid IOP reading was digitally displayed. Measurements by the Tono-pen were made 3 times for each time point, and the mean value was calculated. Care was taken to avoid too much pressure on the cornea. If the IOP decreased significantly after application of the Tono-pen, the data were discarded. After the surgical operation, each rabbit was allowed to recover for 2 h, during which IOP reached a constant value.

**Protocol**

**Experiment 1: Comparison of IOP measurement by the needle insertion technique and the Tono-pen tonometry.** In this group of animals ($n=6$), another 25-gauge needle was inserted into the anterior chamber of the left eye and connected to a bottle filled with artificial aqueous humor (Opeguard MA, Takeda). The IOP was altered by changing the height of the bottle. At the beginning, the height of the bottle was maintained where IOP measured by the needle insertion technique showed 10 mmHg. The height was then raised to each of six positions above the left eye: 20.3, 27.0, 33.8, 40.5, 54.0, and 67.5 cm. The measurements with Tono-pen were performed 3 min after the change in height.

**Experiment 2: Relationship between tilt angle and IOP.** At the beginning of the experiment, each rabbit ($n=6$) was placed at 75° head-up tilt (HUT) position. The angle was changed to 60, 45, 30, 15, and 0° (horizontal) and tilted head-down at 15, 30, 45, 60, and 75°. The animal was then returned to the 75° HUT position at intervals of 15°. Tilt speed was approximately 3%/s. The animals were maintained at each angle for 10 min. The IOP measured by the pressure transducer was averaged over 30 s from 4.5 to 5 min after the change in angle. The measurements of
IOP with the Tono-pen were performed after 5 min. 

**Experiment 3: Changes in IOP before, during, and after 8 h of HDT at 45°**. After baseline measurements were obtained for 1 h at the horizontal prone position, the animals \(n=6\) were tilted head-down at 45° for 8 h and then returned to the horizontal prone position for the next 1 h of the recovery period. The IOP was measured continuously with the needle insertion technique, and the data were averaged for 30 s at the following time points: immediately (within 3 min) and every hour after the onset of HDT, and immediately (within 3 min) and 1 h after the end of HDT. The IOP measurements using the Tono-pen were obtained at 1 h and also at 5 min before HDT; at 5 min and every hour after the onset of HDT, and at 5 min and 1 h after the end of HDT. In the control experiment, the rabbits \(n=6\) remained in the horizontal prone position for 10 h. The collection of data was made at the same times as the HDT experiment.

**Data analysis.** Values in the text, figures, and tables (except for Table 1) are given as means±SEM, and \(n\) is the number of rabbits. A linear regression curve was obtained for comparison of IOP values between invasive and noninvasive techniques. The difference in means was analyzed by a nonpaired t-test between HDT and control groups at each time. The difference was considered to be significant when \(p<0.05\).

**RESULTS**

Measurements of IOP using the Tono-pen were always performed by one (A.S.) of the authors. Reproducibility of the method was examined in six rabbits in Experiment 1 (Table 1).

### Table 1. Reproducibility of IOP measurements with Tono-pen.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>1st IOP (mmHg)</th>
<th>2nd IOP (mmHg)</th>
<th>3rd IOP (mmHg)</th>
<th>Mean±SD (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13.0±0.0</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>12.3±0.6</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>16</td>
<td>15</td>
<td>15.3±0.6</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>12.3±0.6</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>12.3±0.6</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>14.0±1.0</td>
</tr>
</tbody>
</table>

**Fig. 1.** Typical recording of IOP<sub>NEEDLE</sub> during the change in height of the bottle from 13.5 to 20.3 cm above the left eye.

### Table 2. Change in IOP<sub>NEEDLE</sub> resulting from alteration of the bottle height.

<table>
<thead>
<tr>
<th>Bottle height (cm)</th>
<th>Expected IOP (mmHg)</th>
<th>Measured IOP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5</td>
<td>10.0</td>
<td>10.8±0.2</td>
</tr>
<tr>
<td>20.3</td>
<td>15.0</td>
<td>15.1±0.1</td>
</tr>
<tr>
<td>27.0</td>
<td>20.0</td>
<td>20.5±0.1</td>
</tr>
<tr>
<td>33.8</td>
<td>25.0</td>
<td>25.2±0.1</td>
</tr>
<tr>
<td>40.5</td>
<td>30.0</td>
<td>30.1±0.1</td>
</tr>
<tr>
<td>54.0</td>
<td>40.0</td>
<td>40.3±0.1</td>
</tr>
<tr>
<td>67.5</td>
<td>50.0</td>
<td>50.6±0.1</td>
</tr>
</tbody>
</table>

IOP, intraocular pressure; IOP<sub>NEEDLE</sub>, IOP measured by needle insertion technique.

**Table 2.** Change in IOP<sub>NEEDLE</sub> resulting from alteration of the bottle height.

IOP<sub>NEEDLE</sub> monitored by the catheter insertion technique changed rapidly and reached a constant value within a minute (Fig. 1). The value remained constant for the rest of 5 min, and a significant reduction in IOP resulting from leakage was not observed. The IOP value increased by the amount expected from the calculation of hydrostatic pressure because of the height of the bottle (Table 2). The measurements obtained by the Tono-pen tonometry showed smaller values of IOP than those obtained by the needle insertion technique. However, a significant correlation \((r=0.99)\) was observed between IOP values measured with the pressure transducer (IOP<sub>NEEDLE</sub>) and the Tono-pen (IOP<sub>TONO-PEN</sub>). Linear regression analysis yielded this equation: IOP<sub>TONO-PEN</sub> = 0.67 IOP<sub>NEEDLE</sub> - 0.67 (Fig. 2).

### 2. Relationship between tilt angle and IOP

The IOP<sub>NEEDLE</sub> reached a new steady-state value within 3 min after the tilt angle had been changed. The average value of IOP in 6 rabbits was 9.5±1.5 mmHg at 75° HUT position: this increased to 13.7±1.1 mmHg at the horizontal prone (0°), to 18.0±1.5 mmHg at 45° HDT, and to the maximum value of 20.9±1.9 mmHg at 75° HDT (Fig. 3). During increasing tilt, IOP decreased depending on the tilt angle. After returning to 75° HUT, IOP (8.4±
Fig. 2. Relationship between intraocular pressure (IOP) measured by the needle insertion technique (IOP$_{NEEDLE}$) and the Tono-pen tonometry (IOP$_{TONO-PEN}$) during changes in the hydrostatic pressure ($n=6$).

$$Y = 0.67X - 0.67$$
$$R = 0.99$$

Fig. 3. Changes in intraocular pressure (IOP) measured by the needle insertion technique (IOP$_{NEEDLE}$, ○) and the Tono-pen tonometry (IOP$_{TONO-PEN}$, □) during postural change ($n=6$). Vertical bars indicate standard error of the mean. HDT, head-down tilt; HUT, head-up tilt.

1.4 mmHg) was not significantly different from the initial value at 75° HUT. Tono-pen tonometry showed a similar change in IOP, but the slope of the angle-IOP curve was different between the two methods. During tilting from the horizontal prone (0°) to 75° HDT, the changes in IOP were 7.3±0.8 and 4.4±1.3 mmHg when IOP was measured with the pressure transducer and the Tono-pen, respectively (Fig. 4).

3. Changes in IOP before, during, and after 8 h of HDT at 45°

Table 3 shows femoral mean arterial pressure and arterial CO$_2$ tension before, during, and after exposure to 8 h of HDT. The arterial pressure decreased during HDT because of hydrostatic effect and returned toward the pre-HDT baseline level during post-HDT recovery. The CO$_2$ tension did not change significantly throughout the experiment.

Figure 5a demonstrates the time course of IOP measured by the needle insertion technique in the control ($n=6$) and HDT ($n=6$) groups. In the control experiments, the IOP decreased gradually during the time course of 10 h. In the HDT group, the pre-HDT baseline value of IOP was not significantly different from that in the control group. The IOP elevated from 13.1±1.3 to 16.9±1.0 mmHg immediately after the onset of HDT and then gradually declined. The values of IOP during HDT were significantly higher than the values in the control group at all time points. The IOP was decreased immediately after returning from HDT to the horizontal prone position and approached the value in the control group. Similar results were obtained with the measurement by the Tono-pen (Fig. 5b). The values of IOP during HDT ($n=6$) were higher than the values in the control group ($n=6$) except at one point (4 h after the onset of HDT). An increase in IOP right after the onset of HDT was significantly greater ($p<0.05$) when the IOP was measured by the pressure transducer (3.8±0.6 mmHg) than when it was measured by the Tono-pen (1.4±0.4 mmHg) (Fig. 4). The difference in IOP between the HDT group and the control group was always
greater throughout HDT when measured by the pressure transducer than when measured by the Tono-pen.

**DISCUSSION**

The present results demonstrate that changes in IOP induced by applying variable hydrostatic pressure can be detected by both the needle insertion technique and the Tono-pen tonometry in rabbits. The IOP measured by the needle technique responded instantaneously to the alteration of hydrostatic pressure. The increased IOP was maintained for 5 min without significant reduction, suggesting that the insertion of needles did not cause remarkable leakage of aqueous humor at least for 5 min, even at a high IOP level (50 mmHg). Moreover, the obtained value of IOP was very close to the expected value from the hydrostatic pressure as a result of a change in the height of the bottle. Thus, the needle insertion technique is an accurate and useful method to measure IOP for a short period.

Tono-pen tonometry is a noninvasive technique for measuring IOP and has been used in human subjects [15], dogs [16], cats [17], rabbits [18], and rats [19]. The present results demonstrated a good reproducibility, as shown in Table 1. It was also shown that IOP values measured by the Tono-pen (IOP\textsubscript{TONO-PEN}) were lower than those obtained by the needle insertion technique (IOP\textsubscript{NEEDLE}). The absolute value of the IOP\textsubscript{TONO-PEN} was almost two thirds of the IOP\textsubscript{NEEDLE} in all IOP ranges (10–50 mmHg). This is inconsistent with a previous report that the Tono-pen overestimates pressures at low IOP (<15 mmHg) [19]. However, the previous and present studies both showed that the IOP\textsubscript{TONO-PEN} correlated well with the IOP\textsubscript{NEEDLE}. Thus the Tono-pen tonometry is also a reliable method for measuring IOP, although calibration is necessary to obtain absolute values in rabbits.

An angle-dependent alteration in IOP during postural change was demonstrated with both the needle insertion technique and the Tono-pen tonometry. The increase in the IOP\textsubscript{NEEDLE} because of HDT (from 0 to −75°) was significantly greater than the increase in the IOP\textsubscript{TONO-PEN} (Fig. 3). Therefore, the needle insertion technique seems to be better than the Tono-pen tonometry for detecting a small change in IOP during HDT. When the rabbits were tilted from the horizontal prone to the 45° position in Experiment 2, the IOP\textsubscript{NEEDLE} increased about 4.4±0.6 mmHg, which is similar to previous data observed in human subjects exposed to 6–10° HDT [7, 9–12]. Thus we used 45° HDT to investigate the time course of IOP during simulated microgravity in the present study. Variable angles of tail suspension or HDT have been used as an animal model to simulate microgravity. There are, however, no animal experiments, except in our previous report, in which IOP was measured during acute HDT. The present results suggest that 45° HDT may be useful in rabbits to examine change in IOP caused by exposure to microgravity.

Our previous study showed that the elevation of IOP\textsubscript{NEEDLE} immediately after exposure to 45° HDT was 2.2 mmHg in 7 rabbits [13]. The difference between the previous and present experiments is that ocular blood flow was measured simultaneously by means of laser Doppler flowmetry in the previous study. The surgical treatment for measuring ocular blood flow may attenuate the response of IOP to HDT. Another explanation is the difference in the recovery time. In our previous study, the recovery time after surgical treatment was not long enough. The IOP was still high (17.3±1.7 mmHg) at the beginning and was decreasing before exposure to HDT. In the present study, therefore, the animal was allowed to recover for 2 h until IOP reached a steady-state value.

In rabbits, exposure to 45° HDT increases intracra-
nial pressure by more than 10 mmHg [20], which is much greater than the increase in IOP. Venous engorgement in the choroidal tissue is suggested to be responsible for the increased IOP during HDT [7, 12]. We suggested that similar pooling of venous blood would occur in the cerebral tissues during HDT, which would elevate the intracranial pressure [20, 21]. Besides the venous engorgement, a shift of cerebrospinal fluid from the spinal subarachnoid space into the intracranial subarachnoid space seems to play a role in the increase of intracranial pressure. On the other hand, aqueous volume is not likely to change so much immediately after exposure to HDT [7, 12], suggesting that it does not take part in the elevation of IOP.

The IOP increased immediately after the onset of HDT and gradually decreased during the next 8 h of HDT. A sustained increase in IOP during HDT was reported in human subjects, although substantial diurnal variations in IOP were observed during 48 h of HDT [7, 12]. On the other hand, a transient increase in IOP during the first hour of HDT with a subsequent decrease in the second hour was found in humans exposed to 2 h of HDT [6, 8]. In the present study, the IOP value during HDT was higher than in the control group at every time point. Therefore the effect of HDT on IOP seems to continue throughout 8 h of HDT. This is also supported by the finding that IOP decreased to the same level as in rabbits of the control group when an animal was returned from HDT to the horizontal prone position. Thus the gradual decrease in IOP observed during 8 h of HDT does not necessarily mean a fadeaway of the HDT effect. The time-dependent decrease in IOP was observed also in rabbits of the control group. It might be caused by inflammation in the anterior chamber or by microleakage resulting from insertion of a needle or by both. Although these factors are not likely to affect IOP measurements for a short period, they cannot be ruled out when the measurement is continued over several hours. If this is so, the noninvasive technique may be better for measuring IOP during prolonged HDT.

In conclusion, the needle insertion technique and the Tono-pen tonometry are both useful for measuring IOP in rabbits. They can detect changes in IOP resulting from postural change. The needle insertion technique has an advantage over Tono-pen tonometry in measuring IOP for a short period, and vice versa for a prolonged period.

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Intraocular Pressure during HDT


