INTRODUCTION

In the field of neurosurgery, cerebroventricle irrigation and cerebroventricle perfusion therapy are effective therapeutic approaches for treatment of ventriculitis and intraventricular hemorrhage. Cisternal perfusion therapy is conducted as prophylaxis of cerebrovascular spasm after subarachnoid hemorrhage. Furthermore, neurosurgical therapy using neuroendoscopy is considered to be minimally invasive surgery. During these surgical procedures, an artificial cerebrospinal fluid (CSF) prepared aseptically in the hospital; for example, normal saline and/or lactated Ringer’s solution, has long been used for irrigation and/or perfusion. However, the composition of the solutions used to date has the potential to inhibit cerebrospinal function. Although artificial CSF is thought to be identical or very similar to human CSF, there are currently no commercially available formulations for cranial and/or intraspinal irrigation and perfusion.

ARTCEREB®, irrigation and perfusion solution (Artcereb), an artificial pharmaceutical, is typically applied inside the skull and spinal cavity as artificial fluid. Artcereb is composed of glucose and electrolytes (Na⁺, K⁺, Mg²⁺, Ca²⁺, Cl⁻, HCO₃⁻ and P) and has a pH of 7.3. An in vitro assessment of the effects of Artcereb on cell culture of rat fetal astrocytes or rat fetal brain cells was performed in comparison with normal saline and lactated Ringer’s solutions. Furthermore, the effects of Artcereb on cell culture of rat fetal brain cells were also assessed in comparison with Krebs bicarbonate solution. Cell function after exposure to Artcereb was assessed based on ³H-thymidine incorporation activity. Cell function after exposure to Artcereb and lactated Ringer’s solution in primary cultures of rat fetal astrocytes remained unaffected when compared to that after exposure to normal saline. Cell function after exposure to Artcereb in a primary culture of rat brain cells remained unaffected as compared to that after exposure to normal saline and lactated Ringer’s solution. However, function decreased after exposure to a modified Artcereb formulation lacking bicarbonate, thus confirming that the presence of bicarbonate is essential for the Artcereb formulation.

Key words: ARTCEREB®, Cerebrospinal surgery, Irrigation solution, Perfusion solution
solution have been reported when a large volume of perfusate was used in place of CSF (Griffith, 1986; Griffith and Jamjoom, 1990). After endoneurosurgery for symptomatic aqueductal stenosis, patients who received normal saline as perfusion fluid developed headaches, high fever and neck stiffness, but patients who received artificial CSF experienced only slight fever (Oka et al., 1996).

We found that Artcereb is unlikely to induce cellular toxicity, while normal saline and/or lactated Ringer’s solution is likely to induce cellular toxicity, based on cell cultures of rat brain cells (Nishimura and Naito, 2008; Nishimura et al., 2010). Using cell cultures of human astrocytes, Artcereb showed no tendency to induce cellular toxicity when used as perfusate in place of saline solution and/or lactated Ringer’s solution (Enomoto et al., 2004; Nishimura et al., 2009). Furthermore, we showed no morphological central nervous system changes with Artcereb (Doi et al., 2009), thus suggesting that Artcereb is a more effective irrigation fluid than both normal saline and lactated Ringer’s solution, and that Artcereb minimized postoperative brain edema and cellular damage during experimental neurosurgery in rats (Doi et al., 2006).

The purposes of present study were to compare 3H-thymidine incorporation and lactate dehydrogenase (LDH) activities after exposure to Artcereb, normal saline or lactated Ringer’s solution using passage cultures of rat fetal astrocytes and primary cultures of rat fetal brain cells, and to assess the influence of these solutions on cell function. We also evaluated the effects of bicarbonate in the study solutions on brain cells and examined the consequences of pH changes caused by varying bicarbonate or CO2 concentrations, as pH increases (some of which may be transferred into other chambers) during the use of Artcereb are predicted.

### MATERIALS AND METHODS

#### Materials
Dulbecco’s MEM medium and fetal bovine serum were purchased from DS Pharma Biomedical Co., Ltd. (Osaka, Japan). Rat fetal astrocytes and astrocyte growth medium Bulletkit® were purchased from Lonza Walkersville, Inc. (Walkersville, MD, USA). Penicillin-streptomycin solution, amphotericin B, and hydroxyurea were purchased from Sigma Aldrich (St. Louis, MO, USA). [3H]-Thymidine was purchased from MP Biomedicals, LLC (Santa Ana, CA, USA). ACS-II was purchased from GE Healthcare UK, Ltd. (Buckinghamshire, England). Normal saline

#### Table 1. Composition and pH levels of study solutions

<table>
<thead>
<tr>
<th>Component</th>
<th>Normal human CSF[a]</th>
<th>Normal rat CSF[b]</th>
<th>Artcereb</th>
<th>Kreb’s bicarbonate solution</th>
<th>Lactated Ringer’s solution</th>
<th>Normal saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mEq/l)</td>
<td>145.5</td>
<td>150-152</td>
<td>145</td>
<td>154</td>
<td>130</td>
<td>154</td>
</tr>
<tr>
<td>K⁺ (mEq/l)</td>
<td>2.8</td>
<td>3.5-6.2</td>
<td>2.8</td>
<td>2.9</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Mg²⁺ (mEq/l)</td>
<td>2.2</td>
<td>2.6-4.8</td>
<td>2.2</td>
<td>2.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca²⁺ (mEq/l)</td>
<td>2.3</td>
<td>3.0-5.0</td>
<td>2.3</td>
<td>4.7</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Cl⁻ (mEq/l)</td>
<td>111.9</td>
<td>132-136</td>
<td>129</td>
<td>134.1</td>
<td>109</td>
<td>154</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/l)</td>
<td>23.1</td>
<td>24-26</td>
<td>23.1</td>
<td>26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SO₄²⁻ (mEq/l)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>-</td>
<td>2.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P (mmol/l)</td>
<td>1.1</td>
<td>n.a.</td>
<td>1.1</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactate (mEq/l)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>-</td>
<td>-</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Glucose (g/l)</td>
<td>0.61²</td>
<td>0.72-1.8</td>
<td>0.61</td>
<td>1.90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Osmolality ratio</td>
<td>about 1</td>
<td>n.a.</td>
<td>about 1</td>
<td>about 1.1</td>
<td>about 0.9</td>
<td>1</td>
</tr>
</tbody>
</table>

(289 mOsm/kg H₂O)

| pH              | 7.307               | n.a.              | about 7.3 | about 7.4                    | about 6.7                 | about 6.3     |


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(Otsuka Normal Saline), lactated Ringer’s solution (Lactec Injection), Kreb’s bicarbonate solution, and Artcereb were supplied by Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). All other reagents were of commercially available analytical grade.

**Animals and diets**

Female Sprague-Dawley rats at 10 weeks of age and at 10 days gestation were obtained from Charles River Japan, Inc. (Kanagawa, Japan). Rats were housed in a room with a 12-hr light:dark cycle and were allowed free access to water and standard rat diet containing (per 100 g) 54.5 g starch, 22.4 g protein, 5.7 g fat, 3.1 g fiber, and 6.6 g mineral and vitamin mixture (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan). The study was approved by the Committee on the Care and Use of Laboratory Animals of Otsuka Pharmaceutical Factory, Inc.

**Cell culture of rat fetal astrocytes**

Passage and monolayer cultures of rat fetal astrocytes were performed according to the instruction manual from Lonza Walkersville, Inc. Cells were used for experiments at 6-7 days after inoculation.

**Cell culture of rat fetal brain cells**

Rats at 15-17 days gestation were used for the preparation of fetal rat brains cells. Monolayer cultures of fetal rat brains cells were prepared as described previously (Nishimura and Naito, 2008). Fetal rat brains cells (containing astrocytes, neurocyte, and microglia) were used for experiments at 6-7 days after inoculation.

**Exposure to various perfusates in rat fetal astrocytes**

Cells were washed and then exposed to normal growth medium (control), normal saline, lactated Ringer’s solution, or Artcereb for 1 or 3 hr under 5% CO₂ in air at 37°C. After each exposure, perfusates were replaced with fresh growth medium and cells were incubated for 0 or 24 hr under 5% CO₂ in air at 37°C (Fig. 1).

**Exposure to various perfusates in rat fetal brain cells**

Cells were first washed and exposed to normal growth medium (control), normal saline, Kreb’s bicarbonate solution, or Artcereb for 3 hr under 5% CO₂ in air at 37°C. Cells were then washed and exposed to normal growth medium (control), normal saline, normal saline with 19 mEq/l bicarbonate (HCO₃⁻), lactated Ringer’s solution, lactated Ringer’s solution with 19 mEq/l HCO₃⁻, Artcereb, or Artcereb without HCO₃⁻ for 1 or 3 hr under 5% CO₂ in air at 37°C. Next, cells were washed and exposed to normal growth medium (control), normal saline, or modified Artcereb (HCO₃⁻ concentrations of 0-135.0 mEq/l) for 3 hr under 5% CO₂ in air at 37°C. Finally, cells were washed and exposed to normal growth medium (control),

![Fig. 1](image-url)
normal saline, lactated Ringer’s solution, or Artcereb for 3 hr under 0.1-17.6% CO₂ in air at 37°C. After each exposure, perfusates were replaced with fresh growth medium and cells were incubated for 0 or 24 hr under 5% CO₂ in air at 37°C (Fig. 1).

**Incorporation of 3H-thymidine**

After incubation for 0 or 24 hr, both ³H-thymidine (final level of 0.037 MBq) and hydroxyurea (final level of 1 mM) were added to growth medium, and cells were incubated for 2 hr under 5% CO₂ in air at 37°C. Cells were washed three times with ice-cold PBS, 10% ice-cold trichloroacetate was added, and cells were incubated for 1 hr at 4°C. Cells were then washed twice with ice-cold PBS, 0.5 N NaOH was added, and cells were incubated for 2 hr at 37°C. Lysates were transferred into a vial and neutralized with 0.5 N HCl, after which 8 ml ACS-II was added. The radioactive content in each vial was determined using a Liquid Scintillation Analyzer 2500 TR (PerkinElmer Life And Analytical Sciences, Inc., Waltham, MA, USA).

**LDH activity**

After incubation for 0 or 24 hr, cells were washed twice with ice-cold PBS, and 0.1% Triton X-100 was added. Samples were analyzed for LDH using an autoanalyser (7170, Hitachi High-Technologies Co., Tokyo, Japan). LDH activity was measured by the Japan Society of Clinical Chemistry (JSCC) using L type Wako LDH J (Wako Pure Chemicals, Osaka, Japan).

**Statistical analysis**

Results are given in terms of percent relative to controls, and values are means ± S.D. Statistical analysis was performed by paired Student’s t-test (two-tailed) with a significance level of p < 0.05.

**RESULTS**

Artcereb is applied directly to exposed sites at the time of brain surgery. As astrocytes and nerve cells, etc., are directly exposed to Artcereb, we evaluated the effects on these cells. Therefore, we used ³H-thymidine uptake as a marker of cell proliferation, and the intracellular LDH activity as marker of cytotoxicity. Furthermore, as Artcereb includes bicarbonate, we also performed an examination regarding the importance of bicarbonate. Astrocytes greatly contribute to nutrient supply, export of metabolite products, and protection of nerve cells in the brain. We thus employed fetal astrocytes in the present study. In addition, because cells such as astrocytes, nerve cells and endothelial cells are exposed to irrigation solution in neurosurgery, we also used fetal brain cells in this study.

**Effects of exposure to Artcereb, lactated Ringer’s solution or normal saline in passage cultures of rat fetal astrocytes**

A comparison of ³H-thymidine incorporation activity in passage cultures of rat fetal astrocytes after exposure to Artcereb, lactated Ringer’s solution or normal saline is shown in Fig. 2. The ³H-thymidine incorporation activities immediately after 1- or 3-hr exposure to Artcereb, lactated Ringer’s solution or normal saline were comparable to those of cells in normal cultures. However, the ³H-thymidine incorporation activity at 24 hr after 3-hr exposure to normal saline was significantly lower compared to exposure to Artcereb, while activity after 3-hr exposure to lactated Ringer’s solution was comparable to exposure to Artcereb. A comparison of intracellular LDH activity in passage cultures of rat fetal astrocytes after exposure to Artcereb, lactated Ringer’s solution or normal saline is shown in Fig. 3. The results for intracellular LDH activity were similar to those for ³H-thymidine incorporation activity.

**Effects of exposure to Artcereb, Kreb’s bicarbonate solution or normal saline in passage cultures of rat fetal brain cells**

A comparison of ³H-thymidine incorporation activity in passage cultures of rat fetal brain cells after exposure to Artcereb, Kreb’s bicarbonate solution or normal saline is shown in Fig. 4. The ³H-thymidine incorporation activities immediately after 3-hr exposure to Artcereb was comparable to those of cells in normal cultures. On the other hand, the ³H-thymidine incorporation activities immediately after 3-hr exposure to Kreb’s bicarbonate solution or normal saline were significantly lower when compared to normal cultures or to exposure to Artcereb. The ³H-thymidine incorporation activity at 24 hr after 3-hr exposure to normal saline was significantly lower when compared to normal cultures or to exposure to Artcereb, while activity at 24 hr after 3-hr exposure to Artcereb or Kreb’s bicarbonate solution were comparable to those of cells in normal cultures.

**Effects of bicarbonate in primary cultures of rat fetal brain cells**

A comparison of ³H-thymidine incorporation activity in primary cultures of rat fetal brain cells after exposure to Artcereb, normal saline or lactated Ringer’s solution is shown in Fig. 5. The effects of bicarbonate in the study solutions on brain cells are also shown in Fig. 3.
The $^3$H-thymidine incorporation activity of cells exposed to Artcereb was comparable to that of cells in normal cultures. However, in the Artcereb formulation lacking bicarbonate, significant reductions in activity were observed at 24 hr after 1- and 3-hr exposure. On the other hand, the $^3$H-thymidine incorporation activities were low immediately after 3-hr exposure to normal saline (8%) and lactated Ringer’s solution (46%), and decreased further 24 hr after exposure. When sodium bicarbonate (19 mEq/l $\text{HCO}_3^-$) was added to normal saline or lactated Ringer’s solution, the $^3$H-thymidine incorporation activity was increased.

Fig. 2. Evaluation of damage to the cell nucleus on passage cultures of rat fetal astrocytes. Cell activity was assessed based on $^3$H-thymidine incorporation in triplicate wells. Data are shown as percent vs. control in normal culture medium. Values are means ± S.D. (n = 3). Statistical significance of differences between values was analyzed by Student’s $t$ test (two-tailed). Significant differences at *$p < 0.05$ vs. Artcereb group, and †$p < 0.05$, and ††$p < 0.01$ vs. Normal culture medium group.

Fig. 3. Evaluation of intracellular LDH activity on passage cultures of rat fetal astrocytes. LDH activity was measured in triplicate wells. Data are shown as percent vs. control in normal culture medium. Values are means ± S.D. (n = 3). Statistical significance of differences between values was analyzed by Student’s $t$ test (two-tailed). Significant differences at †$p < 0.05$, and ††$p < 0.01$ vs. normal culture medium group.
solution, the $^3$H-thymidine incorporation activity of brain cells remained higher than that when solutions lacked bicarbonate, but was generally lower than when the Artcereb formulation was used.

**Effects of pH changes caused by bicarbonate concentration in primary cultures of rat fetal brain cells**

A comparison of $^3$H-thymidine incorporation activity after exposure to regular Artcereb, Artcereb with various bicarbonate concentrations to alter pH, or normal saline is shown in Fig. 6. After 3-hr exposure to normal saline (pH 4.5) under 5% CO$_2$ gas in a CO$_2$ incubator, $^3$H-thymidine incorporation into the cells decreased to 26%. In the case of Artcereb lacking bicarbonate, in which pH was 4.8 under 5% CO$_2$, the incorporation activity immediately after or 24 hr after exposure tended to be lower than that with the regular formulation (pH 7.4). When the pH of the Artcereb formulation was adjusted to 6.8-8.2 by varying sodium bicarbonate concentrations, no influence was observed in the $^3$H-thymidine incorporation activity of brain cells immediately after or 24 hr after 3-hr exposure to the test solution, and the activities at all pH levels remained at similar levels to those for normal culture medium (MEM).

**Effects of pH changes caused by CO$_2$ concentration in primary cultures of rat fetal brain cells**

A comparison of $^3$H-thymidine incorporation activity after exposure to MEM, Artcereb, or normal saline formulations with pH changes caused by varying incubator CO$_2$ concentrations (0.1-17.6%) is shown in Fig. 7. The $^3$H-thymidine incorporation activity with MEM at different pH levels varied little immediately after and 24 hr after 3-hr exposure. In the saline solutions, the $^3$H-thymidine incorporation activity at each pH level decreased substantially after 3-hr exposure, and the decrease in activity at lower pH was particularly marked. Furthermore, after exposure to saline at pH levels above 5.0, the $^3$H-thymidine incorporation activity was elevated 24 hr after exposure, indicating some recovery of cell activity. However, the $^3$H-thymidine incorporation activity at pH levels below 4.5 24 hr after exposure mostly disappeared. With regard to Artcereb in pH ranges above 7.4, the cell activities of $^3$H-thymidine incorporation were relatively maintained compared to control levels with normal MEM. Although the $^3$H-thymidine incorporation activity of cells immediately after 3-hr exposure to Artcereb at pH 6.9 under 17.6% CO$_2$ decreased significantly to 59%, it recovered to normal levels 24 hr after replacing the medium with MEM.
Fig. 5. Effects of bicarbonate on primary cultures of rat fetal brain cells. Cell activity was assessed based on 3H-thymidine incorporation in duplicate wells. Sodium bicarbonate (19 mEq/l HCO\textsubscript{3}\textsuperscript{-}) was added to normal saline or lactated Ringer’s solution. Data are shown as percent vs. control in normal culture medium (MEM). Values are means ± S.D. (n = 5). Statistical significance of differences between values was analyzed by Student’s t test (two-tailed). Significant differences at *p < 0.05, **p < 0.01 and ***p < 0.001 vs. Artcereb group, and †p < 0.05, ††p < 0.01, and †††p < 0.001 vs. Normal culture medium group.
Fig. 6. Effects of pH changes caused by bicarbonate concentration on primary cultures of rat fetal brain cells. Cell activity was assessed based on $^3$H-thymidine incorporation in duplicate wells. Data are shown as percent vs. control in normal culture medium (MEM). Values are means ± S.D. (n = 5). Statistical significance of differences between values was analyzed by Student’s $t$ test (two-tailed). Significant differences at **$p < 0.01$ and ***$p < 0.001$ vs. pH 7.4 (Artcereb) group.
Fig. 7. Effects of pH changes caused by CO₂ concentration on primary cultures of rat fetal brain cells. Cell activity was assessed based on ³H-thymidine incorporation in duplicate wells. Data are shown as percent vs. control in normal culture medium (MEM). Values are means ± S.D. (n = 3). Statistical significance of differences between values was analyzed by Student’s t test (two-tailed). Significant differences at * p < 0.05 and **p < 0.01 vs. 5% CO₂ concentration.
DISCUSSION

Artcereb is typically used as an artificial fluid for applications inside the skull and spinal cavity. To investigate its effects on human brain tissue, we have been using cell cultures of rat fetal brain cells and human fetal astrocytes, and based on morphological and biochemical studies, determined the usefulness of Artcereb when compared to saline and lactated Ringer’s solutions (Nishimura and Naito, 2008; Enomoto et al., 2004). Cell morphology in cultures of rat fetal brain cells exposed to Artcereb showed no differences from that in normal cultures (Nishimura and Naito, 2008). In contrast, reductions in cell number and cell transformation in rat fetal brain cells were observed after exposure to normal saline and lactated Ringer’s solution (Nishimura and Naito, 2008). The various uptake activities of rat fetal brain cells after exposure to Artcereb were significantly higher than those after exposure to saline solution and lactated Ringer’s solution (Nishimura and Naito, 2008), indicating the protection of brain cell function by Artcereb. In addition, the mitochondrial activity of rat fetal brain cells was not affected by Artcereb exposure (Nishimura and Naito, 2008). We also found that exposure of human fetal astrocytes to Artcereb brought about no morphological condensation of nuclear chromatin and slight changes in histone phosphorylation, unlike the cell nuclei with condensed chromatin and marked changes in histone phosphorylation observed after exposure to saline and lactated Ringer’s solutions (Enomoto et al., 2004). Thus, exposure to Artcereb appears to have little effect on brain cell function and is associated with little induction of apoptotic cell death.

Kreb’s solution is often used in experiments employing cells. A comparison between Artcereb and Kreb’s bicarbonate solution was thus performed. 3H-thymidine incorporation activities after exposure to Kreb’s bicarbonate solution decreased immediately after exposure as compared to exposure to Artcereb. This may have been due to differences in electrolyte composition and glucose concentrations between the solutions. However, as the reasons for this difference remain uncertain, further investigation into the mechanisms is necessary.

In the present study, the Artcereb formulation was pharmacologically evaluated based on 3H-thymidine incorporation activities in primary cultures of rat brain cells in order to elucidate the implications of both bicarbonate constituents and pH level of Artcereb. Rat brain cells exposed to Artcereb showed no decreases in 3H-thymidine incorporation activity, while cell activity decreased markedly after exposure to saline or lactated Ringer’s solution. However, modified Artcereb lacking bicarbonate resulted in significantly reduced cell activity under standard culture conditions with 5% CO₂, indicating that the presence of bicarbonate is essential for the formulation. Although the exposure of brain cells to normal saline or lactated Ringer’s solution markedly reduced 3H-thymidine incorporation activity, cell activity was notably higher when 19 mEq/l bicarbonate (HCO₃⁻) was added to the solution. To further determine the effects of Artcereb pH, modified formulations with pH 6.8-8.2 were prepared with various concentrations of sodium bicarbonate (6.3-135 mEq/l) and were then exposed to brain cells. Modified Artcereb solutions showed no substantial influence on 3H-thymidine incorporation activity of cells, similarly to the results obtained in previous experiments on mitochondrial activity (Nishimura and Naito, 2008). In addition, it was confirmed that the pH changes in Artcereb, derived by varying the incubator CO₂ concentrations (0.1-5.0%), had little effect on 3H-thymidine incorporation into brain cells, except at pH 6.9 (under 17.6% CO₂). The decreased thymidine incorporation activity after 3-hr exposure to Artcereb at pH 6.9 under a high CO₂ atmosphere, however, recovered 24 hr after replacing the medium with MEM. When pH was adjusted by changing the sodium bicarbonate concentrations, modified Artcereb had no effect at pH 6.8. Therefore, the decreased cell activity at pH 6.9 under 17.6% CO₂ may have been due to the higher CO₂ concentration, as compared to standard conditions (5.0% CO₂). These results suggest that the bicarbonate in Artcereb is an essential factor for maintaining cellular function, and the buffering system based on the equilibrium between sodium bicarbonate and carbonic acid may play an important role in preventing damage to brain cells. However, even when bicarbonate is added to normal saline, 3H-thymidine uptake is not comparable to that with Artcereb (Fig. 5). Moreover, we previously reported that the presence of both bicarbonate and Mg²⁺ is important for the Artcereb formulation (Nishimura et al., 2010). Therefore, although cellular protection increases in the presence of bicarbonate, other ions are considered to be essential.

In conclusion, the results of the present study demonstrate that function in primary cultures of rat brain cells remains normal after exposure to Artcereb, in contrast to function after exposure to normal saline or lactated Ringer’s solution, thus suggesting the efficacy of Artcereb formulation as an irrigation and perfusion solution in the field of neurosurgery.
REFERENCES


