Development of Artificial Cerebrospinal Fluid: Basic Experiments, and Phase II and III Clinical Trials

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Abstract

Objectives: The first artificial cerebrospinal fluids (ACSFs), Elliot’s solutions A and B, were first reported in 1949. In 1974, we developed an ACSF, ACSF trial product No. 1, and have safely used and developed this ACSF since then (No.1 to No.19). In parallel with this, after performing basic experiments and clinical trials, we obtained marketing approval for a commercially available ACSF (ARTCEREB) from the Japanese Ministry of Health, Labor, and Welfare in 2008. We now present the results of those basic experiments and clinical studies, including the phase II and III trials on ARTCEREB, with special reference to its safety and efficacy.

Methods: The composition and properties of ARTCEREB are as the same as human CSF, excluding the Cl concentration. Experiments with rats: the significance of bicarbonate in ACSF, and the influence and cytotoxicity of ARTCEREB, lactated Ringer’s solution, and normal saline were assessed in cultured brain cells derived from rat fetuses by examining mitochondrial activity and morphological changes. In rats with brain injury, we examined the influence of these three irrigation and perfusion fluids on edema, vascular permeability, and mitochondrial activity. Clinical trials: we performed the phase II and III clinical trials on 157 patients in 17 institutions from 2002 through 2005.

Results: Experiments with rats: the morphological changes observed in the ARTCEREB-treated group were less than in the other two groups. Mitochondrial activity was higher in the ARTCEREB group than observed for the other two solutions. Mitochondrial activity in the ARTCEREB, lactated Ringer’s solution, and normal saline groups was approximately 10%, 50%, and 70%, respectively, lower than observed in intact cultured brain cells. There was a significant reduction of edema and vascular permeability at the injured site in the ARTCEREB group compared to the other two groups. Mitochondrial activity was significantly higher in the ARTCEREB group than in the other two groups, indicating the lower cytotoxicity of ARTCEREB. Clinical trials: the only adverse effects associated with the use of ARTCEREB were a slightly increased temperature in two patients and decreased alkaline phosphatase activity in two patients, indicating the safety and efficacy of ARTCEREB.

Conclusions: In the present study, basic experiments on cultured brain cells derived from rat fetuses and in the injured rat brain, combined with phase II and III clinical trials demonstrated the safety and efficacy of ARTCEREB.

Keywords: Artificial cerebrospinal fluid; Irrigation fluid; Minimally invasive surgery; Neuroendoscopic surgery; Perfusion fluid

Abbreviation: ACSF: Artificial cerebrospinal fluid; CSF: Cerebrospinal Fluid; ARTCEREB: Brand Name of the Artificial Cerebrospinal Fluid; EB: Evans Blue; NIH: National Health Insurance; osm prss: Osmotic Pressure; TTC: 2,3,5-Triphenyltetrazolium Chloride; WNL: Within Normal Limits; Pharmaceutical Manufacturer: Otsuka Pharmaceutical Factory, Inc, Tokushima, Japan.

In 1949, Elliott [1] reported the first artificial cerebrospinal fluids (ACSFs), Elliot’s solutions A and B. Although 60 years have passed since then, ACSFs are still not widely used because the development cost is too high and there is a low recognition of the usefulness of ACSFs by neurosurgeons. In 1974, we developed the ACSF hospital trial product No.1 in the Department of Neurosurgery, School of Medicine, Keio University, Japan. Since then, this ACSF has been continually developed up to trial product No.19 and these products were used until 2008. During this 34-year period, these ACSFs have been safely used on 10,000 patients undergoing neurological surgery [2-6].

In parallel with this, we have developed an ACSF product, ARTCEREB, in collaboration with a pharmaceutical manufacturer, and conducted a variety of studies on ARTCEREB including basic experiments [7,8], studies on its use as an irrigation fluid for neurosurgery [9,10] and in phase II [11] and III [12] clinical trials. In 2008, the Japanese Ministry of Health, Labor, and Welfare approved the marketing of ARTCEREB (NHI drug price listing). It is expected that ARTCEREB will be extensively used worldwide in the fields of neurosurgery and neurosciences.

Methods

Artcereb

The safety and efficacy of ARTCEREB were comparatively studied with other irrigation and perfusion fluids, e.g., lactated Ringer’s solution and normal saline, in basic experiments, and in phase II and III clinical trials. The composition and properties of ARTCEREB are shown in Table 1, and the composition of human CSF reported by Davson [13] and Milhorat [14] is shown in Table 1. Na+ 145.4 (145.5) mEq/L, K+ 2.8 (2.8) mEq/L, Mg2+ 2.2 (2.2) mEq/L, Ca2+ 2.3 (2.3) mEq/L, CI 128.5 (111.9) mEq/L, HCO3- 23.1 (23.1) mEq/L, P 1.1 (1.1) mmol/L, glucose

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Rhodamine 123 and microscopically observed 24 hours after exposure to solutions for 3 hours. The mitochondria were fluorescently stained with rhodamine 123 which is easily incorporated into cells [15]. Cultured brain cells were exposed to the test solutions specifically distributed to the mitochondria, and fluorescently stains the corresponding component in human CSF (defined as 100).

ARTCEREB is contained in a double compartment bag to suppress the interaction of the constituents; the bag is further packed within a CO₂ barrier free outer bag to maintain the pH stability. To use ARTCEREB, lactated Ringer’s solution is infused or dripped from the outer bag and the septum is opened by manual compression to mix the two fluids. Although ARTCEREB was infused or dripped into the operative field, its pH was kept within the allowable range (below pH 7.8) when stored in an open system, such as a bowl, for 6 hours at room temperature (Figure 2).

Animal preparation

This study was approved by the Otsuka Pharmaceutical Factory Committee on the Care and Use of Laboratory Animals and was conducted in accordance with in-house guidelines that follow the Guide for the Care and Use of Laboratory Animals (US National Research Council). Two sets of basic experiments were conducted in rats.

Table 1: Composition of test solution and human normal CSF.

| Component | Unit | Human normal CSF | ARTCEREB | Lactated Ringer’s solution |
|-----------|------|------------------|----------|---------------------------|
| Na⁺       | mEq/L| 145.5            | 144.5    | 154                       | 130.0 |
| K⁺        | mEq/L| 2.8              | 2.8      | ----                      | 4.0   |
| Mg²⁺      | mEq/L| 2.2              | 2.2      | ----                      | ----  |
| Ca²⁺      | mEq/L| 2.3              | 2.3      | ----                      | ----  |
| Cl⁻       | mEq/L| 111.9            | 128.5    | 154                       | 109.0 |
| HCO₃⁻      | mEq/L| 23.1             | 23.1     | ----                      | 28.0  |
| P         | mmol/L| 1.1              | 1.1      | ----                      | ----  |
| Glucose   | g/L  | 0.61             | 0.61     | ----                      | ----  |

Artificial cerebrospinal fluid (ARTCEREB) contains lactate (28 mEq/L), which is less than lactate in human normal cerebrospinal fluid (HCSF). pH values should be maintained within the permissible range (pH 7.3 ± 0.2). The rat brain, an approximate 1 g organ, contains approximate 1 mosm/kg of P₃O₅ (7.307). (Table 1). Figure 1 shows the relative concentration of each component in ARTCEREB, lactated Ringer’s solution, and normal saline to the corresponding component in human CSF (defined as 100).

Figure 1: Ratio of composition of solutions to human normal CSF. Lactated Ringer’s solution contains lactate (28 mEq/L) instead of HCO₃⁻ (23.1 mEq/L) in ARTCEREB.

Evolution of the significance of bicarbonate in irrigation and perfusion fluids on the mitochondrial activity of cultured brain cells derived from rat fetuses

A primary culture system of rat fetus brain cells [8] was used. Brains were isolated from rat fetuses on the 15-16th day of pregnancy under ether anesthesia. A suspension of viable cells, approximately 1.0 × 10⁵ cells/mL, was prepared from the brain tissue. Cell preparations in which the viability was higher than 90% were used in the experiments. The cell suspension was dispensed into a 6-well plate in aliquots of 2 mL/well or into a 24-well plate in aliquots of 0.4 mL/well. The cells were cultured in a 5% CO₂ incubator. The culture medium was exchanged on the 3rd and 5th days, and cells were used on the 6th to 7th day after plating.

Rhodamine 123 which is easily incorporated into cells is specifically distributed to the mitochondria, and fluorescently stains these organelles [15]. Cultured brain cells were exposed to the test solutions for 3 hours. The mitochondria were fluorescently stained with rhodamine 123 and microscopically observed 24 hours after exposure (Figure 3). In addition, the amount of rhodamine 123 incorporated into the cultured cells was measured and we calculated the relative amount of rhodamine 123 in cells exposed to the test solutions with a comparison to the intact cultured cells (Figure 4).

Cellular damage and edema in injured rat brains

We used 88 Sprague-Dawley rats, weighing 290-300 g (Charles River Japan, Inc., Yokohama, Japan) to study brain edema, cerebrovascular permeability and mitochondrial activity of damaged brain tissue. Animals were randomly allocated to the normal saline group, the lactated Ringer’s solution group, or the ARTCEREB group. Rats were anesthetized by the intraperitoneal injection of urethane, and were positioned in a stereotactic frame (SR-6N; Narishige Scientific Instrument Laboratory, Tokyo, Japan). A burr hole opening (4 mm diameter), the removal of the dura mater and arachnoid membrane, and a 1.5 mm depth and 3.5 mm length wound were made. The wounds were separately irrigated with 150 mL/hr of the 3 test fluids for 4 hours. The irrigation solutions were administered in a blinded manner. The rats were kept warm with a feedback-controlled lamp and a warming pad to maintain a rectal temperature of approximately 37°C until the end of the irrigation period (Figure 5).

Part 1: determination of the specific gravity of brain tissue: As the irrigation period, the rats were killed by exsanguination from the abdominal aorta, and the brains were quickly removed. To prevent evaporative water loss, we temporarily stored each brain in cooled container.

![Figure 2](image-url)
Marmarou et al. [16]. The column was calibrated with potassium sulfate (Wako Pure Chemical Industries, Ltd) of a known density, following the method of (Wako Pure Chemical Industries, Ltd) and kerosene (Wako Pure Chemical Industries, Ltd, Osaka, Japan) until the gravity values were read and recorded accordingly.

The samples were placed in a gravimetric column (2). Part 2: Two percent of EB in saline was given intravenously 3 hours after the injury (3). Four hours after injury, irrigation was stopped. To remove the intravascularly localized dye. After decapitation, the brain was removed and examined for evidence of EB staining, and then separated into cortices and basal ganglia. Cortices were flattened on a plate, and, using a 4-mm-diameter cork borer, brain tissue samples were obtained from the injured and uninjured sites. The extraction and determination of the EB dye were performed according to the modified method reported by Uyama et al. [17] Brain samples were weighed and homogenized in a 10-fold volume of phosphate buffer and mixed with a 10-fold volume of 50% trichloroacetic acid to precipitate the proteins. The supernatant was obtained by centrifugation and diluted 4-fold with ethanol. Fluorescence was determined (excitation at 620 nm and emission at 680 nm) with a spectrofluorometer (FP-750, JASCO Co, Tokyo, Japan). The calculations were based on external standards of the solvent (25-500 ng/mL). The tissue content of EB was expressed as micrograms per gram of brain tissue. Rats in the control group were administered with EB 3 hours after body temperature maintenance was initiated, and they underwent the same operation as did the other groups.

Part 3: TTC incubation and measurement of tissue formazan levels: 2,3,5-Triphenyltetrazolium chloride (TTC) is reduced in surviving tissue by mitochondrial succinate dehydrogenase to a red formazan product. TTC staining of brain tissue followed by solvent extraction and spectrophotometric measurement of formazan can, therefore, provide an objective index of experimental brain injury. We performed TTC staining and measurement of tissue formazan...
levels in brain tissue according to the modified method reported by Preston and Webster [18]. The irrigation was stopped 4 hours after the injury. The animals were decapitated, and their heads were chilled on ice for approximately 30 seconds. The brains were removed and separated into cortices and basal ganglia. Cortices were flattened on an ice-cooled plate, and, using a 4-mm-diameter corksor, brain tissue samples were obtained from the injured and uninjured sites. Each tissue sample was cut in half, and weighed in pre-tared liquid scintillation vials. A bathing solution, containing 140 mmol/L sodium chloride, 5 mmol/L potassium chloride, 1 mmol/L calcium chloride, 10 mmol/L HEPES, and 3 mmol/L glucose, was used to dissolve the TTC (2% solution), and 5 mL was added to each tissue vial. The vials were incubated for 90 minutes at 37°C. The TTC was then removed, and the tissue was rinsed twice with normal saline. Approximately 5 g of a 50:50 mixture of ethanol/dimethyl sulfoxide was added to solubilize the formazan. The vials were tightly capped and placed in the dark for 24 hours. For analysis, the red solvent extract was placed in a cuvette and the absorbance of this cuvette was measured at 485 nm in a spectrophotometer (V-550, JASCO Co).

Statistical analysis

The experimental data are expressed as the mean ± SD. Statistical analysis was performed using Dunnett’s test for multiple comparisons to evaluate the intergroup differences. Differences were considered to be statistically significant at P<0.05.

Clinical trials

The clinical trials were conducted as non-randomized and non-blind open studies according to the ethical principles of the Declaration of Helsinki [19] (Helsinki, Finland, June 1964), the standards provided by Article 14 Paragraph 3 and Article 80 Paragraph 2 of the Japanese Pharmaceutical Affairs Law (August 10, 1960: Law No. 145) [20], and the Ordinance of the Ministry of Health and Welfare for the implementation standards for the clinical tests of drugs (March 27, 1997: Ordinance of the Ministry of Health and Welfare, Japan, No. 28) [21].

Phase II clinical trial [11]: Although lactated Ringer’s solution and normal saline have been used for intracranial irrigation, intraventricular perfusion, and infusion during neurological surgery, such usage has not been officially approved in Japan. Since this clinical trial was the first trial in which ARTCEREB was applied to humans, the trial was separated into two steps and performed as follows: step 1 was sequentially carried out in patients undergoing mild, moderate, and severe surgical invasion in a stepwise manner to confirm the safety of ARTCEREB; and step 2 was carried out with no thought to the severity of the surgical invasion.

Patients who met any of the criteria listed in Table 2 were excluded from the study. We studied 44 patients aged between 25 and 75 years in 3 institutions. Forty patients underwent neurological surgery with a burr hole opening and craniotomy, and four patients underwent neuroendoscopic surgery. Safety was evaluated based on the primary and secondary evaluation endpoints shown in Table 3. Collected data were analyzed from the medical viewpoint in comparison with clinical survey samples. To determine the clinical efficacy (performance) of ARTCEREB in practical use, we evaluated: (1) its capacity to irrigate an operative field, (2) its capacity to exclude air from the operative field, and (3) its influence on a surgical coagulation device (electric coagulator) in patients undergoing neurological surgery with a burr hole opening and craniotomy. We also assessed: (1) its capacity to secure the cleanliness of the operative field, (2) the adhesive feeling of the perfusion fluid, and (3) its influence on a surgical coagulation device in patients undergoing neuroendoscopic surgery.

Phase III clinical trial [12]: Patients who met any of the exclusion criteria (Table 2) were excluded. A safety evaluation was performed in 113 patients in 17 institutions for the endpoints shown in Table 3. Subjects included 98 patients who underwent neurological surgery with a burr hole opening and craniotomy, and 15 patients who underwent neuroendoscopic surgery. In addition, we surveyed the clinical efficacy (performance) of ARTCEREB in practical use among the surgeons for the same items used in the phase II clinical trial. Adverse events and judgment of adverse effects: any unfavorable

| Table 2: Subjects of the clinical study (Exclusion criteria). [Modified and translated with permission from reference [11]]. |
|----------------------------------------------------------|
| Primary endpoint Clinical symptoms endpoint other than primary endpoints |
| Axillary temperature | Clinical symptoms |
| Headache | Neurological findings |
| Nausea | Vital signs |
| Vomiting | Hematological examination |
| Consciousness (JCS) | Blood biochemical examination |
| Blood Cl concentration |

Table 3: Endpoints for safety evaluation. JCS=Japan Coma Scale [33,3]; [Modified and translated with permission from reference [11]]. |
or unintended signs, symptoms, or diseases (including abnormal laboratory values) associated with the use of ARTCEREB were considered as adverse events. An adverse event whose causal relationship with the use of ARTCEREB could not be refuted was judged to be an adverse effect.

Data collection and statistical analysis: the efficacy of ARTCEREB was evaluated by the frequency distribution categorized into four levels. Data were collected on the frequency distribution, adverse events and their incidence, the incidence of intracranial complications among adverse events, and the degree and frequency of systemic complications. Descriptive statistics were calculated for indiscrete values.

Results

Evaluation of the influence of the irrigation and perfusion fluids on the mitochondrial activity of cultured brain cells derived from rat fetuses

Microscopic observation of cultured brain cells derived from rat fetuses, which were fluorescently stained with rhodamine 123, revealed that there were no differences in the amount of incorporated rhodamine123 in the cells and mitochondria of the ARTCEREB group and intact cultured cells. On the other hand, the lactated Ringer’s solution and normal saline groups showed decreased rhodamine 123 incorporation into cells and mitochondria, a decreased number of cells, and more severe morphological changes in viable cells in comparison with the ARTCEREB group (Figure 3). Cultured cells treated with ARTCEREB, which contained bicarbonate and a pH adjusted to 7.4, showed higher rhodamine 123 uptake than the lactated Ringer’s solution and normal saline groups; the mitochondrial activity in the lactated Ringer’s solution and normal saline groups was 45% and 32%, respectively, lower than observed in the intact cultured cells (Figure 4). In addition, although the mitochondrial activity of cells exposed to ARTCEREB was similar to that of intact cultured cells 24 hours after exposure, the mitochondrial activity of cells exposed to bicarbonate-free ARTCEREB was significantly lower than that observed in the intact cultured cells.

Specific gravity of injured brain tissue

The specific gravities of the injured sites were significantly lower in the normal saline (1.025 ± 0.001) and lactated Ringer’s solution (1.031 ± 0.002) groups than in the ARTCEREB group (1.035 ± 0.003; P<0.001 and P<0.01, respectively). The specific gravities of the uninjured sites in the ARTCEREB (1.049 ± 0.001), lactated Ringer’s solution (1.048 ± 0.001), and normal saline (1.049 ± 0.001) groups were similar, but they were lower than that observed in the injured sites (Figure 4). In addition, although the mitochondrial activity of cells exposed to ARTCEREB was similar to that of intact cultured cells, the specific gravities of the uninjured sites in the control (0.245 ± 0.017) and ARTCEREB (0.246 ± 0.017) groups were not statistically different (Figure 8).

TTC staining of injured brain tissue

TTC staining of the injured sites did not differ significantly between the control (0.242 ± 0.017) and ARTCEREB (0.220 ± 0.023) groups. TTC staining of the injured sites in the lactated Ringer’s solution (0.189 ± 0.023) and normal saline (0.168 ± 0.030) groups was significantly lower than in the control group (P<0.001 for each). TTC staining of the injured sites in the lactated Ringer’s solution and normal saline groups was significantly lower when compared to the ARTCEREB group (P<0.05 and P<0.01, respectively). TTC staining of the uninjured sites in the control (0.244 ± 0.014), ARTCEREB (0.254 ± 0.020), lactated Ringer’s solution (0.237 ± 0.016), and normal saline (0.232 ± 0.018) groups was not statistically different (Figure 9).

Clinical trials: phase II and phase III

The backgrounds of the study subjects of phase II and phase
III clinical trials are shown in Table 4. The volume of ARTCEREB administered in the phase II clinical trial [11] was 100-4130 mL (mean ± SD: 1237.1 ± 922.5 mL) and 1850-4000 mL (mean ± SD: 2527.5 ± 1014.7 mL) for patients undergoing neurosurgery with a burr hole opening and craniotomy, and those undergoing neuroendoscopic surgery, respectively. In the phase III clinical trial [12], 50-8750 mL (mean ± SD: 1283.6 ± 1164.5 mL) and 350-2980 mL (mean ± SD: 1376.0 ± 813.2 mL) of ARTCEREB were used for patients undergoing neurosurgery, respectively. In the phase III clinical trial [12], 50-8750 mL (mean ± SD: 1283.6 ± 1164.5 mL) and 350-2980 mL (mean ± SD: 1376.0 ± 813.2 mL) of ARTCEREB were used for patients undergoing neurosurgery with a burr hole opening and craniotomy, and those undergoing neuroendoscopic surgery, respectively (Table 4).

Evaluation of safety: a total of 2,745 mild, moderate, and severe adverse events were reported in patients undergoing neurological surgery with a burr hole opening and craniotomy and those undergoing neuroendoscopic surgery. We observed decreased alkaline phosphatase activity in two patients undergoing neurological surgery with a burr hole opening and craniotomy, and a slightly increased temperature in two patients (37.4°C and 38.1°C, respectively) who underwent neuroendoscopic surgery. We observed decreased alkaline phosphatase activity in two patients undergoing neurosurgery with a burr hole opening and craniotomy, and those undergoing neuroendoscopic surgery, respectively. In the phase III clinical trial [12], 50-8750 mL (mean ± SD: 1283.6 ± 1164.5 mL) and 350-2980 mL (mean ± SD: 1376.0 ± 813.2 mL) of ARTCEREB were used for patients undergoing neurosurgery, respectively (Table 4).

Figure 8: Evans blue concentration of brain tissue 4 hours after injury. Data are expressed as mean ± SD. Statistical analysis was performed using Dunnett’s test for multiple comparisons to evaluate intergroup differences. [Modified with permission from reference [7]].

Figure 9: Staining with TTC (absorbance per milligram of protein of brain tissue) of brain tissue 4 hours after injury. Data are expressed as mean ± SD. Statistical analysis was performed using Dunnett’s test for multiple comparisons to evaluate intergroup differences. [Modified with permission from reference [7]].

Adverse events were judged to be a slight increase in temperature, and although it was considered to be an adverse effect because it was an unusual postoperative event, it was not considered that the changes were medically significant. The slightly increased temperature was judged to be an adverse effect by the two principal physicians because the possibility of chemical (aseptic) meningitis caused by ARTCEREB could not be excluded. The severity of these adverse events was judged to be mild, and all four patients subsequently recovered.

Evaluation of efficacy (performance): the efficacy of each of the assessed items (described in the Methods section) and the overall efficacy were judged to be good or excellent in all patients.

Discussion

Composition of the irrigation and perfusion fluids used in neurological surgery

The use of ACSFs is not yet widespread, despite a considerable number of neurosurgeons and neuroscientists who consider that ACSFs are useful. There are a number of reasons for this observation. First, hospitals that use ACSFs prepare their own as an in-hospital preparation. However, this is expensive and the hospitals have to bear this cost because the use of transfusion fluids has not been approved for intracranial irrigation and perfusion by the Ministry of Health, Labor, and Welfare. Second, pharmaceutical manufacturers have not developed ACSFs as they require a large financial investment and a long research and development period (the development requires approximately 10 years); ACSFs are also not yet profitable as the market size is small. It is considered that ARTCEREB, with a similar composition to human CSF, has become widely used because it obtained marketing approval by the Japanese Ministry of Health, Labor, and Welfare as an irrigation and perfusion fluid.

The composition and properties of ARTCEREB, which we have developed since 1974, are shown in Table 1 and Figure 1. The components and their concentrations of ARTCEREB are the same as those in human CSF except for Cl-; the concentration of Cl- in ARTCEREB is 16.6 mEq/L, which is higher than that in human CSF. Figure 1 shows the relative concentration of each component in ARTCEREB, lactated Ringer’s solution, and normal saline when the concentration of corresponding component in human CSF is defined as 100%. The composition and properties of lactated Ringer’s solution and normal saline are entirely different from those of human CSF. In addition, lactated Ringer’s solution contains lactate instead of the HCO3-. It has been considered that both lactated Ringer’s solution and normal saline are utterly non-physiological and useless, but they have been used as irrigation and perfusion fluids throughout the history of neurological surgery.

Oka et al. [22] reported the occurrence of adverse effects associated with the use of normal saline as an irrigation and perfusion fluid, and suggested that normal saline was not appropriate for this purpose. Kodama et al. [23] did not use normal saline for ventriculo-cisternal perfusion to prevent vasospasm after subarachnoid hemorrhage. Ohira
important because it stabilizes the pH of a solution. On the other hand, solutions (Figures 3 and 4). As pointed out by Lewis, bicarbonate is effects on mitochondrial activity and morphology than the other and perfusion fluids, ARTCEREB had significantly less unfavorable absorption of bicarbonate. In cultured brain cells exposed to irrigation the effects of the irrigation and perfusion fluids on the mitochondrial and perfusion fluids, as early as 1945 and 1950, respectively. We demonstrated that in ACSF as early as 1945 and 1950, respectively. We demonstrated that ARTCEREB, which is more physiological as an irrigation and perfusion fluid, caused less damage to neuronal cells than lactated Ringer’s solution or normal saline [7,8]; our clinical studies demonstrated a favorable safety and efficacy profile of ARTCEREB [11,12]. Therefore, it is considered that ARTCEREB should be more widely used in the field of neurosurgery.

Significance of bicarbonate in ACSF

Elliott [1] and Lewis [31,32] stressed the significance of bicarbonate in ACSF as early as 1945 and 1950, respectively. We demonstrated that the effects of the irrigation and perfusion fluids on the mitochondrial activity of neuronal cells were greatly influenced by the presence or absence of bicarbonate. In cultured brain cells exposed to irrigation and perfusion fluids, ARTCEREB had significantly less unfavorable effects on mitochondrial activity and morphology than the other solutions (Figures 3 and 4). As pointed out by Lewis, bicarbonate is important because it stabilizes the pH of a solution. On the other hand, special care is needed for the preparation and use of ACSF because bicarbonate is labile. The bicarbonate ion, HCO₃⁻, decomposes to H₂O and CO₂ partially when it comes into the water solution; the CO₂ diffuses into the air and the pH of the solution rises. In addition, rises in fluid temperature during steam sterilization under pressure promotes the generation of CO₃²⁻, which binds with Ca²⁺ and Mg²⁺ and further promotes binding of these ions with phosphate ions. These reactions generate insoluble salts, which cause a white clouding with a rise the pH of ACSF.

Therefore, it is evident that bicarbonate is essential in ACSF, but some ingenuity is necessary to prevent the loss of bicarbonate and to stabilize the pH of the solution. This may also be one of the reasons for the delay in the development of a commercially available ACSF. Before ARTCEREB was developed, we (Keio University Hospital) had prepared ACSF with almost the same composition and properties of ARTCEREB by mixing 20 mL (1 ampoule) of Meylon Injection 7% [sodium bicarbonate concentration, 1.4 g/20 mL (7%); pH: 7.9; osmotic ratio: 5] with 500 mL (1 bottle) of ACSF trial product No. 19 just prior to use. To stabilize the bicarbonate, ARTCEREB is packaged in an inner bag which is a double compartment bag, and a CO₂ barrier free outer bag which prevents the escape of CO₂. The inner bag, which is removed from the outer bag just prior to use, is a double compartment bag in which two kinds of solutions are separated by a septum. Both solutions are mixed by opening the septum with manual compression and the mixed solution (ARTCEREB) is administered by a direct drip to the operative field (closed system). ARTCEREB also retained its pH, within the prescribed limit, for 6 hours in an open system (Figure 2). Thus, ARTCEREB can be safely used for longer operations by adding, or by replacing with, fresh solution. ARTCEREB has various improvements for the long-term stabilization of the pH of bicarbonate-containing ACSF, including the adoption of a double compartment bag. No other ACSFs, including Elliott’s solution and inhospital ACSF preparations, have had such great care taken to stabilize the pH [28]. Normal saline has been used instead of an appropriate irrigation and perfusion fluid in Japan (normal saline and Ringer’s solution have only been approved for intravascular injection in Japan). Recently, perfusion fluids approved for the fields of ophthalmology, orthopedics, or urology have also been used as perfusion fluids for surgery.

Basic experiments with rats

We demonstrated that the mitochondrial activity of cultured brain cells exposed to ARTCEREB, measured by the rhodamine123 method, was similar to intact cultured brain cells. However the mitochondrial activity of the lactated Ringer’s solution and normal saline groups were reduced to 45% and 32%, respectively, compared to intact cultured brain cells, indicating that these solutions have unfavorable or damaging effects on these cells [8]. Although the brain tissues around the operative field are injured by surgery, to a greater or lesser extent, the degree of damage in the surrounding tissues, such as edema, increased vascular permeability, and decreased mitochondrial activity, was less in the ARTCEREB group in comparison with the lactated Ringer’s solution and normal saline groups [7]. In other words, ARTCEREB causes milder damage to brain cells compared with the other two solutions. Recently, the concept of minimally invasive surgery has been proposed, not just for neurological surgery but for all surgical fields. Any surgery, at least neurosurgery, which does not use ARTCEREB or ACSF as an irrigation and perfusion fluid cannot be called minimally invasive surgery.

Clinical trials

The purpose of any clinical trial is the ascertainment of safety, which is the most important issue for the clinical use of a new drug. Since the composition and properties of ARTCEREB are very similar to those of human CSF, the occurrence of adverse effects associated with its use is unimaginable. Even if signs or findings of adverse effects appear, it is impossible to attribute their cause to any of the components or properties of ARTCEREB.

However, since ARTCEREB contains bicarbonate, it will release CO₂ and be alkalized when it comes into contact with the air. Thus, it is important to stabilize and maintain the pH during clinical trials and practical clinical use. Therefore, the inner bag of ARTCEREB is packed within a CO₂ barrier free outer bag. Clinical trials of ARTCEREB were conducted with a closed system, and its efficacy (performance) and safety (adverse effects) were judged to be good or excellent in all patients.

There are no grounds for using normal saline or similar perfusion fluids that have harmful effects on neuronal cells for intraventricular perfusion in neuroendoscopic surgery. In addition, even if irrigation and perfusion fluids which have harmful effects on neuronal cells induce no immediate symptoms, the resultant neuronal damage may cause remote symptoms and impairment. It should be emphasized that neurosurgeons who intend to perform minimally invasive surgery should use ARTCEREB, or at least another ACSF, for neurological surgery.

Conclusions

Our basic experiments and clinical trials demonstrated the chemical
stability and safety of ARTCEREB, a commercially available ACSF, in the central nervous system. In particular, the clinical trials proved the excellent efficacy (performance) of ARTCEREB as an irrigation and perfusion fluid in neurological surgery. To improve surgical outcomes in the field of neurological surgery, especially when minimally invasive surgery is intended, ARTCEREB ought to be extensively used.

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References

1. ELLIOTT KA, JASPER HH (1949) Physiological salt solutions for brain surgery; studies of local pH and pial vessel reactions to buffered and unbuffered isotonic solutions. J Neurosurg 6: 140-152.
2. Shiobara R, Toya S, Isilaka Y, Shizawa H, Ichikizaki K (1976) Early surgery of ruptured cerebral aneurysm in the acute stage (Jpn). Annual bulletin of Mitsukoshi health and welfare foundation. Tokyo 12: 37-49.
3. Shiobara R, Toya S, Isilaka Y, Shizawa H, Ichikizaki K (1977) [An evaluation of the continuous ventricular drainage for ruptured cerebral aneurysms (author's trans)]. Neurol Med Chir (Tokyo) 17: 145-152.
4. Shiobara R, Toya S, Isilaka Y, Ootani M, Kawase T (1983) Development and clinical use of artificial cerebrospinal fluid and ventriculo-cisternal irrigation fluid (Jpn). Jpn J Stroke. Proc 8th annual Japanese conference of stroke Tokyo 5: 196.
5. Shiobara R, Kawase T, Toya S, Ebato S, Miyahara Y (1986) Ventriculo-cisternal perfusion using artificial CSF with urokinase for subarachnoid hemorrhage (Jpn). Neurol med chirurg. Proc 45th annual meeting abstracts the Japan neurological society Tokyo 363.
6. Shiobara R, Kawase T, Toya S, Ebato S, Miyahara Y (1985) Scavengery surgery for subarachnoid hemorrhage. II) Continuous ventriculo-cisternal perfusion by use of artificial cerebrospinal fluid with urokinase. In: Auer LM (ed.) Taming of Aneurysm Surgery. Walter de Gruyter Press: Berlin, New York, USA 369-372.
7. Doi K, Kawano T, Morioka Y, Fujita Y, Nishimura M (2006) Various irritation fluids affect postoperative brain edema and cellular damage during experimental neurosurgery in rats. Surg Neurol 66: 565-572.
8. Nishimura N, Doi K, Enomoto R, Lee E, Naito S, et al. (2008) ARTCEREB Irrigation and perfusion solution for cerebrospinal surgery (Jpn). Pharmacological assessment using astrocytes exposed to test solution. Cell Industrial Info Tokyo 40: 254-257.
9. Shiobara R, Ohtera T, Onozuka S, Kawase T (2005) A study of the use of irrigation fluid in neurosurgery (Jpn). Jpn J Neurosurg. Tokyo 14: 323-330.
10. Shiobara R, Ohtera T, Onozuka S, Kawase T (2006) A study of the use of irrigation fluid in neurosurgery II (Jpn). J New Rem & Clin Tokyo 55: 1040-1133.
11. Shiobara R, Kageji T, Nakagawara J, Murakami H, Ohtera T (2007) Safety evaluation of ACF-55 for use as irrigation or perfusion solutions in neurosurgery patients: Phase II clinical trial (Jpn). J New Rem & Clin Tokyo 55: 1454-1456.
12. Shiobara R, Kageji T, Kawase T, Nakagawara J, Ara K, et al. (2007) Multicenter study of ACF-55 for use as irrigation or perfusion solutions in patients who underwent burr hole opening, craniotomy surgery, or neuroendoscopic surgery: Phase II clinical trial (confirmatory study) (Jpn). J New Rem & Clin. Tokyo 56: 1458-1523.
13. Davson H (1967) Chemical composition and secretory nature of the fluid. In: Davson H Physiology of the cerebrospinal fluid. London: J & A Churchill 33-54.
14. Milhorat TH(1972) Cerebrospinal fluid physiology. In: Hydrocephalus and the cerebrospinal fluid. Baltimore USA: Williams & Wilkins 1-41.
15. Johnson LV, Walsh ML, Chen LB (1980) Localization of mitochondria in living cells with rhodamine 123. Proc Natl Acad Sci U S A 77: 990-994.
16. Marmarou A, Poll W, Shulman K, Bhagavan H (1978) A simple gravimetric technique for measurement of cerebral edema. J Neurosurg 49: 530-537.
17. Uyama O, Okamura N, Yanase M, Narita M, Kawabata K, et al. (1988) Quantitative evaluation of vascular permeability in the geldi brain after transient ischemia using Evans blue fluorescence. J Cereb Blood Flow Metab 8: 282-284.
18. Preston E, Webster J (2000) Spectrophotometric measurement of experimental brain injury. J Neurosci Methods 94: 187-192.
19. World Medical Association: Helsinki declaration.
20. The Japanese Government (1960) Article 14, Paragraph 3 and Article 80, Paragraph 2 of Pharmaceutical Affairs Law.
21. Ministry of Health and Welfare, Japan (1997) Ordinance of Ministry of Health and Welfare for implementation standard for clinical tests of drugs.
22. Oka K, Yamamoto M, Nonaka T, Tomonaga M (1996) The significance of artificial cerebrospinal fluid as perfusate and endoneurosurgery. Neurosurgery 38: 733-736.
23. Kodama N, Sasaki T, Kawakami M, Sato M, Asari J (2000) Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm after aneurysmal subarachnoid hemorrhage. Outcome in 217 patients. Surg Neuro 53: 110-117.
24. Ohtera T, Toya S (1996) Rigid-rod neuroendoscopy (Jpn). In: Sato O, Ohi S (eds.) Neuroendoscopic Surgery. Basic knowledge and surgical technique. Tokyo Miwa Shoten 60-64.
25. Ohtera T, Toya S (1996) Navigation endo-microscope (Jpn). In: Sato O, Ohi S (eds.) Neuroendoscopic Surgery. Basic knowledge and surgical technique. Tokyo: Miwa Shoten 101-104.
26. Ohtera T, Kawase T (2001) Complication and countermeasure (Jpn). In: Takakura K, Saito I, Kawase T, Teramoto A (eds.) Neuroendoscopic Surgery. Advanced Practice. Neuroendoscopic Surgery Tokyo Medical View 122-124.
27. Enomoto R, Tatsukoua H, Komai T, Sugahara C, Takemura K, et al. (2004) Involvement of histone phosphorylation in apoptosis of human astrocytes after exposure to saline solution. Neurochem Int 44: 459-467.
28. Japanese Society of Hospital Pharmacists (2003) Artificial cerebrospinal fluid (Jpn), in Japanese Society of Hospital: In-hospital drug preparation. (5th edn), Tokyo Yakujii Nipposha 225-226.
29. Kamikawa H, Kobayashi N (1996) Shunt tube manipulation (Jpn). In: Sato O, Ohi S (eds.) Neuroendoscopic Surgery. Basic knowledge and surgical technique. Tokyo Miwa Shoten 221-225.
30. Oka K, Yamamoto M, Nonaka T, Tomonaga M (2001) Hydrocephalus (Jpn). In: Takakura K, Saito I, Kawase T, Teramoto A (eds.) Neuroendoscopic Surgery: Neurological Surgery. Advanced Practice 3. Tokyo Medical View 60-69.
31. ELLIOTT KA, LEWIS RC (1950) Clinical uses of an artificial cerebrospinal fluid. J Neurosurg 7: 256-260.
32. Oka K, Tomonaga M (1996) Steerable/flexible neuroendoscopy (Jpn). Neuroendoscopic Surgery: Basic knowledge and surgical technique. Tokyo: Miwa Shoten 69-72.
33. Oota T (2000) New classification of level of consciousness (Jpn). In: Oota T, Matsuji K (eds.) Neurosurgery. (8th edn) Kyoto Jpn Kinpoudou 177-183.

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