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CELL TRANSFECTION & CELL FUSION PRODUCTS

Tech-Trends Application Note Volume 5, Series 2

Disruption of Blood Brain Barrier using Electroporation

ABSTRACT

Electroporation, is known to induce cell membrane permeabilization in the reversible (RE) mode and cell death in the irreversible (IRE) mode. Using an experimental system designed to produce a continuum of IRE followed by RE around a single electrode we used MRI to study the effects of electroporation on the brain. Fifty-four rats were injected with Gd-DOTA and treated with a G25 electrode implanted 5.5 mm deep into the striata. MRI was acquired immediately after treatment, 10 min, 20 min, 30 min, and up to three weeks following the treatment using: T1W, T2W, Gradient echo (GE), serial SPGR (DCE-MRI) with flip angles ranging over 5–25°, and diffusion-weighted MRI (DWMRI). Blood brain barrier (BBB) disruption was depicted as clear enhancement on T1W images. The average signal intensity in the regions of T1-enhancement, representing BBB disruption, increased from 1887±83 (arbitrary units) immediately post treatment to 2246±94 20 min post treatment, then reached a plateau towards the 30 min scan where it reached 2289±87. DWMRI at 30 min showed no significant effects. Early treatment effects and late irreversible damage were clearly depicted on T2W. The enhancing volume on T2W has increased by an average of 2.27±0.27 in the first 24–48 hours post treatment, suggesting an inflammatory tissue response. The permanent tissue damage, depicted as an enhancing region on T2W, 3 weeks post treatment, decreased to an average of 50±10% of the T2W enhancing volumes on the day of the treatment which was 33±5% of the BBB disruption volume. Permanent tissue damage was significantly smaller than the volume of BBB disruption, suggesting, that BBB disruption is associated with RE while tissue damage with IRE. These results demonstrate the feasibility of applying reversible and irreversible electroporation for transient BBB disruption or permanent damage, respectively, and applying MRI for planning/monitoring disruption volume/shape by optimizing electrode positions and treatment parameters.

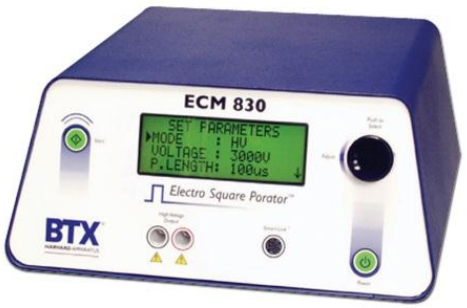
METHODS

Intracranial Electrode Placement

The bregma was identified through a midline scalp incision, and one 1 mm burr hole was drilled in the right or left region of the skull, 3 mm anterior and 2 mm lateral to the bregma. 25-gauge stainless-steel electrodes were placed stereotactically in the striatum at a depth of 5.5 mm. A second, large 4 cm by 8 cm flat electrode was pressed against the rat chest after applying conducting gel for better electric coupling. The electrodes were connected to the pulse generator. Control rats Underwent similar procedures, including electrode implantation, without applying the electric pulses.

Electroporation Treatment Protocol

Rats were treated using a conventional electroporator power supply (BTX 830; Harvard Apparatus, Holliston, MA). Voltages used in the experiments ranged from 250 V to 650 V, the number of pulses ranged from 50 to 90, the pulse duration ranged from 50 ms to 70 ms, and the pulse delivery frequency was 4 Hz.



ECM 830 Square Wave Generator Cat. #45-0052

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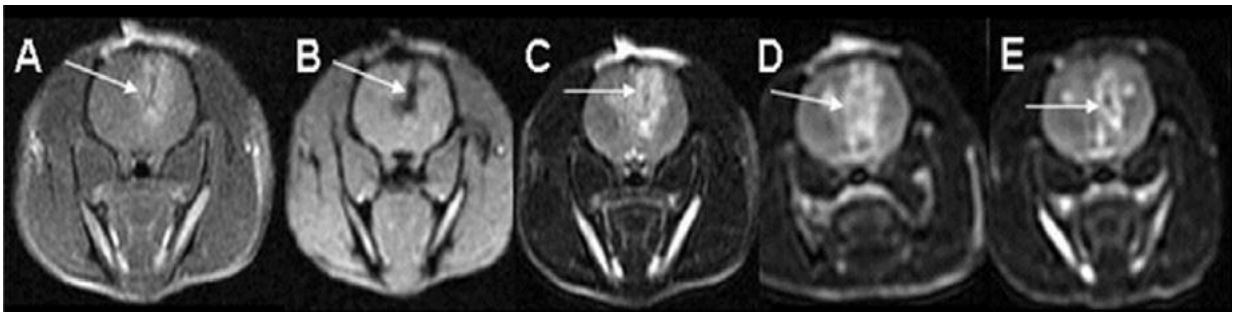

MRI sequences for depicting electroporation effects in the rat brain.
T1W (A), Gradient-echo (B) and T2W (C–E) MRI of a rat treated with one intracranial electrode and another external flat electrode pressed against the rat chest. Treatment was performed with 50 pulses of 650 V, 70 µs duration and a frequency of 4 Hz. Significant BBB disruption is depicted as bright enhancement on the T1W images acquired 30 min after treatment (A). The GE image (B) shows signal void along the path of the electrode suggesting hemorrhage. T2W images depict tissue response to the treatment as bright enhancement (C–E). It can be seen that 1 day post treatment (D) the volume of tissue changes seems larger than on the day of the treatment (A), but then the volume is reduced by day 8 (E)

Table 1. Average volumes of BBB disruption and T2-enhancement.

Voltage [V]	# of rats	T1 volume Day 0 [mm^3]	T2 volume Day 0 [mm^3]	T2/T1 volume Day 0	T2 (Day 1–2)/T2 (Day 0)	T2 (3 weeks)/T1 (Day 0)
250	11	24.5±1.6	16.1±2.2	0.67±1.00	1.70±0.41	0.17±0.07
300	6	28.0±3.9	20.5±3.3	0.73±0.06	2.57±0.32	0.21±0.14
350	6	35.2±5.7	26.0±4.9	0.76±0.11	2.70±0.78	0.38±0.09
600	8	109.8±14.1	54.2±10.1	0.57±0.05	3.59±0.78	0.44±0.11
650	5	162.2±29.2	142.7±19.0	0.90±0.07	1.13±0.18	0.57±0.20

*Column 3: Enhancing volume on T1W images at the day of the treatment, representing the volume of BBB disruption (mean±SE).
**Column 4: Enhancing volume on T2W images at the day of the treatment, representing initial tissue response to treatment (mean±SE).
***Column 5: Ratio of enhancing volume on T2W and T1W images on Day 0, showing that BBB disruption volume was always larger than tissue damage volume (mean±SE).
****Column 6: Ratio of enhancing volume on T2W images 24–48 hours post treatment and T2W images on Day 0, showing the tissue response volume significantly increased in the first 2 days post treatment (mean±SE).
*****Column 7: Ratio of enhancing volume on T2-weighted MRI 3 weeks post treatment, representing the permanent tissue damage, and T1-weighted enhancing volume on Day 0, showing that BBB disruption was always larger than the permanent tissue damage (mean±SE).
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RESULTS

Application of thermal reversible electroporation and non thermal irreversible electroporation electric fields to the rat brain demonstrates the feasibility of applying electroporation for significant and transient BBB disruption with and without permanent tissue damage, under real-time MR treatment monitoring and with late MR monitoring of treatment effects. Significant correlation was found between treatment voltage extent of NTRE and BBB disruption volume. BBB disruption volume was significantly correlated with later volume of tissue damage and in all cases depicted a larger volume than the final damage. These results imply that MRI may be used for treatment monitoring of brain electroporation where preservation of healthy tissue is crucial. Furthermore, electroporation may be applied for a combined treatment of systemic chemo + local electroporation, for destruction of brain tumors tissue by IRE, while chemotherapy is efficiently delivered to the surrounding infiltrated tissue due to the larger coverage of temporary BBB disruption.

Hjouj M. et al., MRI Study on Reversible and Irreversible Electroporation Induced Blood Brain Barrier Disruption 2012, *PLoS One*