ACUTE EFFECTS ON BLOOD BARRIER FUNCTION DUE TO HIGH INTENSITY RF-EMF EXPOSURE OF THE RAT BRAIN *IN VIVO*

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ABSTRACT

To explore the biological effects on the blood barrier of radio-frequency electromagnetic field (RF-EMF) exposure, we developed a real-time measuring system during RF-EMF exposure, which was based on a micro-perfusion method and enables the monitoring of intensity changes in fluorescent-labeled albumin concentration from collected cerebrospinal fluid (CSF). Following 1.5 GHz RF-EMF exposure for 30 minutes at 35W/kg (average brain SAR), the fluorescent intensity was increased, suggesting that albumin from circulating blood leaked into CSF due to a blood barrier disorder. However, this effect was not observed in the sham exposure group. The effect was probably evoked by thermal effect due to high intensity RF-EMF exposure. Further study is ongoing.

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INTRODUCTION

Possible health effects of weak RF-EMF have been discussed worldwide. However, further scientific evidences are needed for better health risk assessment, particularly, the effects on the blood-brain barrier (BBB) which are still not clear due to the variety of experimental designs. One of the main problems on BBB study is that there are few methods which are able to monitor the barrier function on real time *in vivo*. In this study, we developed a real-time measuring system for blood-cerebrospinal fluid (CSF) barrier function which defined same as BBB in a broad sense. To collect CSF continuously, we employed micro-perfusion method to the measuring system. We examined high intensity RF-EMF exposure to the rat brain and monitored albumin concentration in CSF during exposure, as a pilot study.

MATERIALS AND METHODS

Animals and surgical procedure

Experiments were performed on male Sprague-Dawley rats weighing between 300 and 400 g. All surgical procedures were performed under sterile conditions. Anesthesia was initiated with a cocktail of ketamine and xylazine (9:1). Rats were set on a stereotaxic instrument, and an incision was made in the rat's scalp to expose coronal sutures. A hole was made at 0.8 mm caudal and 1.3 mm lateral to the sagittal suture. A guide cannula (NG-8FS, Eicom Co., Kyoto) was introduced to one of the lateral ventricles through the hole and secured to the skull with acrylic dental cement. At the end of experiment, Evans blue dye was injected through the cannula into the lateral ventricle to confirm the placement of the cannula.

Experimental protocol

After a 48 hour recovery period, the rats were again anesthetized, and a push pull cannula (NDP-I-8-01FEP, Eicom Co., Kyoto, Japan; Fig.2) was inserted into a guide cannula. The inlet and the outlet of the cannula were connected to the push-pull micro perfusion pump unit (EP-70, Eicom Co.) which enabled the collection of CSF continuously. At the beginning of RF-EMF exposure, FITC-labeled albumin (FITC-albumin) was injected into caudal vein and CSF perfusion was started at a rate of 1 μl/min. Fluorescence intensity of FITC-albumin in perfusate was monitored by using spectrofluorometry (FP-6500, Jasco Co., Tokyo) under the condition of excitation /emission wave length at 490/515nm . Experimental setup was summarized in Fig. 1.

Exposure conditions

Each rat was exposed to RF-EMF (1,439 MHz) at 35W/kg (average brain SAR) for 30 minutes using a loop antenna (Fig.3). In the sham exposure group, rats were kept in an anechoic chamber for 30 minutes without RF-EMF exposure. This exposure conditions are extremely higher than the radiofrequency safety guidelines, i.e., 2 W/kg for the general public. Fluorescence intensity in perfusate was monitored for 180 minutes from the beginning of the exposure.

RESULTS and DISCUSSION:

In this study, we developed a new system, which makes it possible to quantify blood barrier function in vivo with high sensitivity, and can be applied during exposure simultaneously. The fluorescence intensity observed by this system was purely dependent on the concentration of FITC-albumin in perfusate. Therefore, the fluorescence intensity can be recognized as one of the parameters for brain barrier abnormality because, under normal conditions, albumin

concentration in cerebrospinal fluid was physiologically limited and it was about 200 times lower than that in the blood. The fluorescence intensity increase was recognized in the exposure animals but was not in the sham animals. This indicates that blood barrier function was affected by high intense RF-EMF exposure probably due to its thermal effects. More detailed analysis is ongoing in order to clarify whether this effect is only a thermal effect or inherent to electromagnetic exposure.

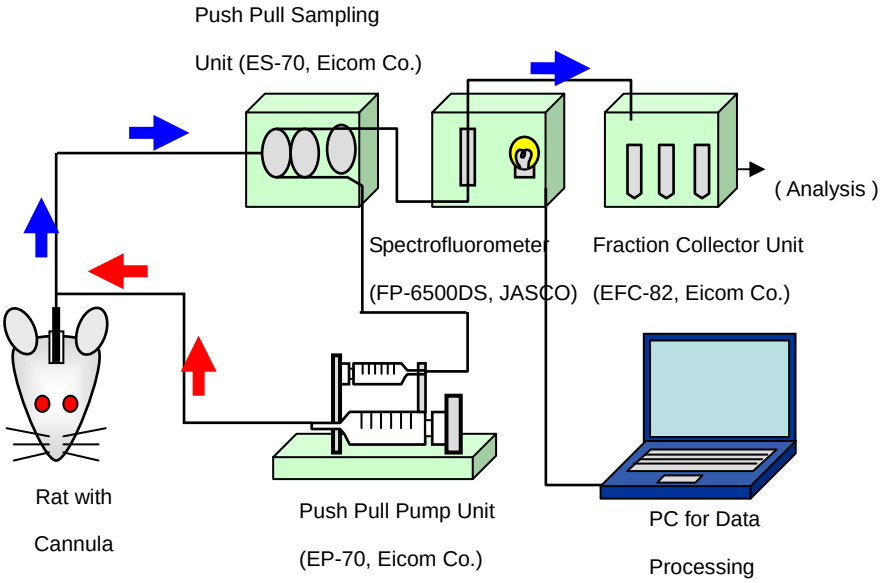

Fig.1 Schematic diagram of developed system for real- time monitoring of blood barrier function

Artificial CSF flow into the lateral ventricle via inlet of cannula (red arrow) and perfusate flow out via outlet of cannula (blue arrow).

Fig 2 Assembly of push-pull cannula for rat

Upper is an inner cannula which includes in/out route and lower is a guide cannula

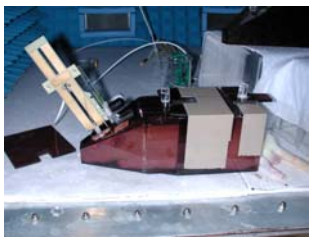

Fig 3 Overview of RF-EMF exposure setup

Rat under anesthesia is fixed in a folder and RF-EMF is exposed by using a loop-antenna.

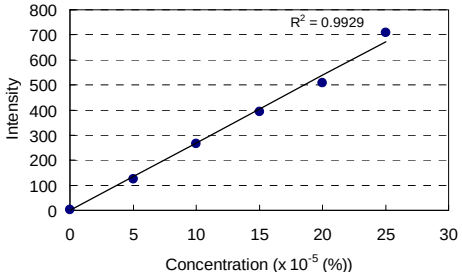

Fig 4 Relationship between the concentration of FITC-labeled Albumin and fluorescence intensity

Observed fluorescence intensity shows linearity against the concentration of FITC Albumin.