CEREBROVASCULAR PERMEABILITY TO ^{14}C-SUCROSE IN THE RAT FOLLOWING 2450 MHz CW MICROWAVE IRRADIATION

[redacted]

To evaluate whether microwaves alter the blood-brain barrier, permeability-area products (PA) have been measured for passage of ^{14}C-sucrose from the bloodstream into brain. Sprague-Dawley rats (450-500 g) were anesthetized with Nembutal and the femoral artery was cannulated. Each rat was placed singly in a prone position in the quiet zone of an anechoic chamber (8x3x3m) with its head facing a horn antenna located 5 m away. Each rat was irradiated with 2450 MHz CW microwaves for 30 min with a power density of either 1 or 10 mW/cm^2, or was sham-irradiated. Linear polarization was used with the E-field vertical. About 10 min after irradiation, 15 μCi of ^{14}C-sucrose was injected intravenously, and small serial samples of arterial blood removed for the next 25 min. After the final blood sample at 25 min, the rat was decapitated and the brain was dissected into 5 regions. Using techniques for liquid scintillation counting, the following values were determined for each rat: C_{brain} (dpm ^{14}C/g for medulla, cerebellum, midbrain, diencephalon and cortex); C_{blood} (dpm ^{14}C/ml for the last sample at 25 min); $\int_0^{25} C_{plasma} dt$ (integral of the plasma level-time curve in which dpm/ml plasma, obtained from blood samples throughout 25 min, were plotted against time). Permeability-area products were calculated from the following equation:[1, 2]
$PA = (C_{brain} - (C_{blood} \times BVS)) \div \int_0^{25} C_{plasma} dt$. The term BVS represents blood volume space in brain (ml/g) and is used to correct C_{brain} for intravascular sucrose. Regional brain BVS values were estimated from separate experiments using the method of reiterative calculations described earlier,[1, 2] and were found to range from 0.0133 to 0.0206. Mean PA values (x10^6) for brain regions from the sham-irradiation group (n=7 rats) ranged from 11.99 ±0.61 (SEM) to 14.47±0.82 sec^{-1}. For the 10 mW/cm^2 group (n=7) mean PA values ranged from 11.96±1.73 to 13.70±1.76 sec^{-1}, and for the 1 mW/cm^2 group (n=5 rats to date) mean PA values ranged from 11.15±0.91 to 12.19±0.56 sec^{-1}. An effect of microwave treatment on barrier permeability cannot be concluded at this point, and the results support negative findings reported earlier in which Brain Uptake Index measurements were used.[3]

[1] Ohno, K. et al. Am J. Physiol. 235: H299-H307, 1978
[2] Rapoport, S.I et al. Brain Research 150; 653-657, 1978
[3] Preston, E. et al. Brain Research (in Press), 1979.

SUMMARY

Rapoport and colleagues[1,2] have described a new radiotracer method for measuring changes in blood-brain barrier permeability in rats. This method has advantages over measurement of Brain Uptake Index (BUI) values, in that it is independent of brain blood-flow changes (which may affect BUI values) and is much more sensitive to barrier perturbations. With the Rapoport method, penetration of a radiotracer such as ^{14}C-sucrose is measured after the tracer has circulated in the blood-stream for half an hour or so, whereas only 15 sec is allowed for penetration of tracer in the BUI method[3]. Using the BUI method we have been unable to detect an increase in barrier permeability to ^{14}C-mannitol in rats which had undergone 30 min irradiation with 2450 MHz microwaves[4]. The following experiments with the more sensitive Rapoport method were undertaken to supplement the brain uptake index experiments.

Male Sprague Dawley rats (450-500 grams) were anesthetized with sodium pentobarbital, and the femoral artery was cannulated. The rat was heparinized and placed singly in a prone position in the quiet zone of a tapered anechoic chamber (8x3x3 m) with its head facing a 10 or 22 dB horn antenna, located 5 m away. Each rat was irradiated for 30 min with a power density of either 1 or 10 mW/cm^2, or was sham-irradiated. 2450 MHz continuous wave power was delivered from a 2 kW Cober generator. Linear polarization was used with the E-field vertical. Chamber temperature was controlled between 21 and 23°C. The probe used in all field measurements was a Holaday Industries Model 1500.

Between 7 and 12 min after irradiation 15 µCi of ^{14}C-sucrose was injected into a femoral vein, and 17 samples of femoral arterial blood of 100 µl volume were taken over the next 25 min. Following the final blood sample taken at the end of 25 min, the rat was decapitated. The brain was dissected into 5 separate regions which were weighed and then digested in NCS solubilizer (Amersham/Searle). All blood samples, except for the last one at 25 min, were centrifuged and 20 µl of plasma removed from each tube. The plasma and blood samples were also digested. 15 ml of ACS fluor was added to all tissue, plasma and blood digests, and after chemiluminescence was minimal, the following values were determined for each rat using liquid scintillation counting: C^{25}brain (dpm ^{14}C/gram) for medulla, cerebellum, midbrain, diencephalon and cortex; C^{25}blood (dpm/ml at 25 min); and dpm/ml plasma from blood samples throughout the 25-min period. For each rat dpm/ml plasma was plotted against time and the integral of the plasma level-time curve determined as $\int_0^{25} C_{plasma} dt$ (dpm.sec/ml). Additional experiments were done in a group of 6 rats which were treated in a manner simulating all procedures carried out on the sham-irradiated group, except that they were decapitated 5 min after intravenous injection of 15 µCi ^{14}C-sucrose, so that most of the tracer measured as C^5brain (dpm/g) could be regarded as intravascular, and the ratio, dpm ^{14}C/g brain: dpm ^{14}C/ml blood (at 5 min), could serve as an initial estimate of blood volume space BVS (ml/g).

Within certain limitations[1] the following relationship can be used to calculate cerebrovascular permeability-area products (PA):

$$PA = \frac{C^{25}\text{brain} - (C^{25}\text{blood} \times BVS)}{\int_0^{25} C_{plasma} dt}$$

The term C^{25}bloodxBVS provides a correction of C^{25}brain for non-parenchymal tracer, i.e. intravascular sucrose. The accuracy of the BVS estimate from 5 min experiments was improved by correcting for the small amount of sucrose which would enter brain within the 5 min period. To do this, mean values of $\int^{25} C_{plasma} dt$, C^{25}brain, and C^{25}blood were calculated for the sham-irradiated rats which had been decapitated 25 min after sucrose. These values were used along with initial BVS estimates from the 5 min experiments to calculate corresponding PA values. These PA values were used with data from the 5 min experiments to calculate parenchymal tracer that had crossed the barrier during 5 min, and thereby an improved, slightly lower blood volume space BVS. These new BVS values was used again with the mean 25 min data to get even more accurate values for PA. Only a few of these reiterative calculations between the 5 min and 25 min experiments were needed for succeeding estimates of BVS to differ by less than 2%, at which point the final BVS estimates were accepted.

Mean values for blood volume space were as follows: medulla--0.0193; cerebellum--0.0206; diencephalon--0.0159; midbrain--0.0185; cortex--0.0133. These mean BVS estimates were used along with all individual values for C^{25}brain, C^{25}blood, and $\int^{25} C_{plasma} dt$ from rats in the sham, 1 mW/cm^2 and 10 mW/cm^2 groups to calculate final PA values.

The accompanying table shows mean PA values (±SEM) for brain regions from 7 sham-irradiated rats, 7 rats irradiated with 10 mW/cm^2, and 5 rats irradiated to date with 1 mW/cm^2. Within each treatment group, the differences between mean PA values for different brain regions are relatively minor. There is also little noteworthy difference in mean PA values between treatment groups; however, PA values in the 10 mW/cm^2 group were more variable as indicated by the standard error values. It must be recognized that changes in brain blood volume and capillary area (A) can account for small variations in PA without permeability (P) necessarily being affected. Also it is known that when the barrier has been opened osmotically, PA increases are large (up to 20-fold)[2]. An effect of microwave treatment on barrier permeability cannot be concluded from the present experiments.

References

[1]Ohno, K., Pettigrew, K.D. and Rapoport, S.I. Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat. Am. J. Physiol. 235: H299-H307, 1978.

[2]Rapoport, S.I., Ohno, K., Fredericks, W.R. and Pettigrew, K.D. Regional cerebrovascular permeability to ^{14}C-sucrose after osmotic opening of the blood-brain barrier. Brain Research 150: 653-657, 1978.

[3]Oldendorf, W.H. Brain uptake of radiolabeled amino acids, amines and nexoses after arterial injection. Am. J. Physiol. 221: 1629-1639, 1971.

[4]Preston, E., Vavasour, E.I. and Assenheim, H.M. Permeability of the blood-brain barrier to mannitol in the rat following 2450 MHz microwave irradiation. Brain Research (in Press), 1979.

Mean Permeability-Area Products (±SEM)

PA x 10^6 (sec^{-1})

30-min

Treatment	medulla	cerebellum	midbrain	diencephalon	cortex
Sham	13.70±0.54	13.02±0.85	13.04±0.66	14.47±0.82	11.99±0.61
1 mW/cm^2	11.15±0.91	11.80±0.86	11.71±0.86	12.19±0.56	11.22±0.74
10 mW/cm^2	11.96±1.73	12.82±1.77	13.43±2.03	13.70±1.76	12.74±1.89