The Relationship of Blood-Brain Barrier Damage to Survival Time After Acute Radiation Injury

Several researchers have demonstrated that functional and morphological blood-brain barrier (BBB) alterations may occur in mammals exposed to high doses of radiation[1-3]. However, the relationship of these alterations to the neurological sequelae and survival time remains to be elucidated. Therefore, a series of studies was performed to test the effects of induced BBB alterations on survival time in rats subsequently exposed to a high dose of radiation.

Materials and methods. Animals. 480 young mature male albino rats weighing 250 ± 20 g from the AFRRI colony were used for these experiments. All animals were given food and water ad libitum, were caged individually and kept at a temperature of approximately 22 °C.

Lymphatic cervical blockade. In the first study, 160 rats were divided into 4 groups of 40. The cervical lymphatic glands were removed according to the procedure of FÖLDI et al.[4] in 2 of the groups (80 rats), in an attempt to produce lymphatic cervical blockade (LCB). 7 days after the surgical procedure, 1 group of 40 rats was exposed to a midline tissue dose of 20,000 rads of mixed γ-neutron radiation, and the other group was sham irradiated.

80 rats were sham operated, and 1 group of 40 was exposed to 20,000 rads of radiation 7 days later. The other group was sham irradiated. The Table lists the experimental groups of rats. One-half of the animals from each group were used to determine survival. Following irradiation, the rats were observed every 15 min; when a death occurred, the group and postirradiation time of death were recorded. This information was used later to plot the survival curves of the various groups of rats. The other half of the animals from each group were sacrificed either at the time of irradiation or 48 h later to test the integrity of the BBB with fluorescein dye. Each rat was first anesthetized with a 36% solution of chloral hydrate injected i.p. (10 ml/kg body wt.) and was then injected i.v. with 0.5 ml of sodium fluorescein (a 5.0% solution of fluorescein, stabilized with 0.24 *M* $NaHCO_3$, adjusted with 0.30 *M* NaOH to pH 9.5)[5]. 30 min after the fluorescein injection, the animals were sacrificed. The brain was perfused with normal physiological saline and then removed. Sections were cut at 2 mm and examined grossly under UV-light to identify the distribution of the fluorescein dye and thus whether or not the BBB was intact.

Glycerol injections. In the second study, 160 rats were divided into 8 groups, in the same manner as described above. In addition, each animal received 50% glycerol (10 ml/kg body wt.) in 2 equal doses. The first injection was given i.m., the second one was given i.p. 10 min later, and 30 min prior to irradiation. This technique has been used by NAIR and ROTH[6] to induce hyperosmolar dehydration and transient BBB alterations.

Mercuric chloride injections. In the third study, 160 rats were divided as in the first study. In addition, each rat was injected i.v. with a solution of mercuric chloride ($HgCl_2$) in saline in a dose of 0.16 m*M*/kg, 24 h prior to radiation exposure.

Irradiation. The rats were irradiated with the AFRRI-TRIGA reactor. A γ-ray field was obtained using 5 inches of water shielding between the reactor core and the exposure room. The γ-ray to neutron tissue kerma ratio was approximately 10. The exposure rate was 2000 rads/min. The total dose was 20,000 rads.

Results. The results of the first survival study are presented in Figure 1. LCB alone induced no deaths. In irradiated rats there was no difference in the 50% mortality time between LCB and sham operated rats. Extravasation of fluorescein was macroscopically evident in the brain slices of both the sham operated and LCB irradiated animals 48 h after exposure. No extravasation of dye was evident in the sham operated or LCB nonirradiated rats.

The experimental groups of rats

Group	Number in group	Description
A	40	LCB, Irradiated
B	40	LCB, Sham irradiated
C	40	Sham operated, Irradiated
D	40	Sham operated, Sham irradiated

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[4] M. FÖLDI, B. CSILLIK and Ö. T. ZOLTAN, Experientia *24*, 1283 (1968).
[5] Moore/Kirk Laboratories, Inc., East Woodstock, Connecticut, USA.
[6] V. NAIR and L. J. ROTH, Radiation Res. *23*, 249 (1964).

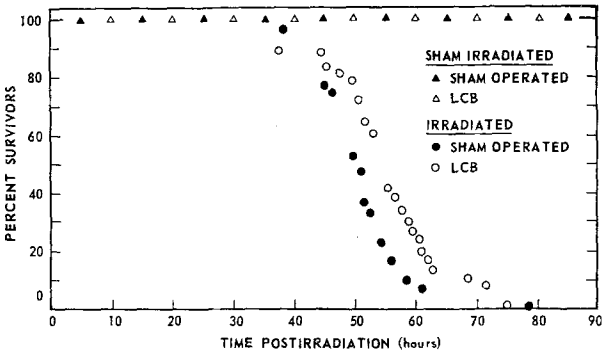

Fig. 1. Survival time of rats with and without lymphatic cervical blockade (LCB) after exposure to 20,000 rads of whole-body mixed γ-neutron radiation.

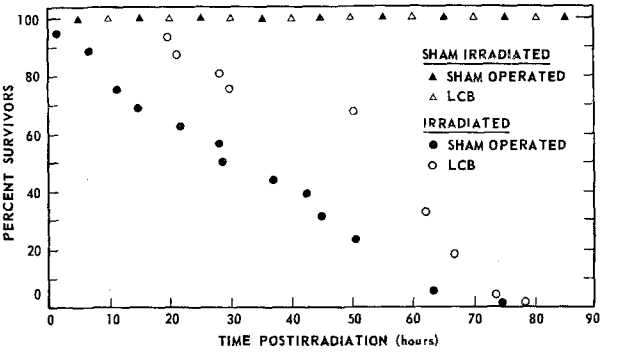

Fig. 2. Survival time of glycerol pretreated rats with and without lymphatic cervical blockade (LCB) after exposure to 20,000 rads of whole-body mixed γ-neutron radiation.

In glycerol treated rats, the survival of the irradiated sham operated rats was nearly linear with time (Figure 2). 50% mortality was reached approximately 32 h post-irradiation, and all rats died within 75 h. The 50% mortality time of the LCB group was approximately 57 h after exposure, but all rats in this group were dead 79 h after exposure. Extravasation of fluorescein was macroscopically visible in the brain slices taken in all 4 groups involved in this study at the time of irradiation, and in the LCB and sham operated irradiated groups 48 h following exposure. No deaths occurred in the nonirradiated glycerol treated LCB or sham operated rats.

Rats in the third study showed very pronounced BBB alterations 24 h after receiving $HgCl_2$ injections as indicated by the extravasation of fluorescein dye. The 50% mortality time of the irradiated LCB and sham operated rats did not differ significantly from each other (Figure 3). Again, fluorescein extravasation was visible in the brain slices taken from all groups 48 h after irradiation especially in the $HgCl_2$ treated LCB animals. No deaths occurred in the nonirradiated $HgCl_2$ treated LCB or sham operated rats.

Discussion. Among the reported findings that result from a supralethal dose of irradiation are neurotransmission disorders[7,8], cerebral capillary injury[1,9,10], and BBB alterations[1,2,9,10]. Even though BBB alterations are seen following central nervous system radiation injury, it is not clear whether these alterations per se appreciably affect survival time.

In this study, a dose of 20,000 rads of whole-body γ-neutron radiation was selected which resulted in 50% mortality within 48 h. 3 different techniques to alter the BBB prior to irradiation, which produced a wide range of damage to the BBB, were used: lymphatic cervical blockade[4], glycerol injection[6] and $HgCl_2$ injection[11]. Extravasation of injected fluorescein into the brain parenchyma confirmed that these 3 methods caused BBB alterations without causing the death of the animal.

The BBB alterations thus induced by LCB and glycerol or $HgCl_2$ injection, when followed by irradiation, did not significantly shorten survival when compared to animals receiving irradiation alone. This suggests that BBB alteration prior to irradiation does not contribute significantly to mortality, further suggesting that BBB damage may bear no direct relationship to survival after acute radiation injury.

Résumé. Les altérations de la barrière sang-cerveau avant l'irradiation n'a pas influencé le temps éventuel de survivance des rats exposés aux doses supermortelles de radiation. Ce résultat suggère la possibilité qu'il n'existe pas de rapport direct entre le dommage de la barrière sang-cerveau et le temps de survivance après irradiation.

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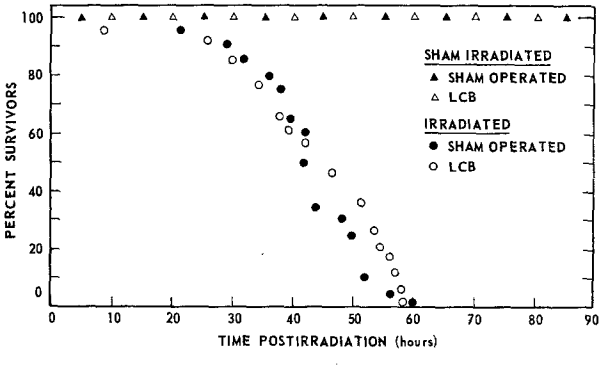

Fig. 3. Survival time of mercuric chloride pretreated rats with and without lymphatic cervical blockade (LCB) after exposure to 20,000 rads of whole-body mixed γ-neutron radiation.

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A Renal Tumour of the Nephroblastoma Type in *Praomys (Mastomys) natalensis*

The *Mastomys* or *Praomys natalensis* is described by OETTLE[1] as an intermediate rodent between the rat and the mouse. Different tumours develop spontaneously in this animal. The most known is a malignant argyrophilic carcinoïd of the stomach (SNELL et al.[2]).

Material and methods. 10 couples of *Mastomys*, aged $1^1/_2$ months and weighing from 40–50 g, were supplied by the Medical Research Council, National Institute for Medical Research of London in May 1971. They were reproduced in our laboratory. We weighed them systematically every 2 weeks and when one *Mastomys* showed abnormal signs as loss of weight, decrease in motility, palpation of an abnormal mass, it was sacrified.

Our study is directed towards *Mastomys* aged from 17–24 months and weighing from 50–80 g. 10 *Mastomys* died without us being able to take specimens from the organs; all were females. For histological study, paraffin sections were cut at 7 µm and stained with Hemalun-Erythrosine-Safran. Our histoenzymatic study regarding the cholinesterase activities in the different organs of the *Mastomys* followed our usual scheme (DELBARRE et al.[3]).

Results. Macroscopic examination (Table). 14 *Mastomys* were sacrified. One had a vegetating and infiltrating cutaneous tumour of 20 mm × 20 mm in diameter which showed the histological picture of differentiated squamous cell carcinoma of the epidermis.

5 Mastomys had renal tumours, unilateral in 3 cases and bilateral in 2. In *one Mastomys* with a unilateral renal

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