Acta Radiologica Oncology 21 (1982) Fasc. 4

FROM THE GUSTAF WERNER INSTITUTE, UPPSALA UNIVERSITY, S-751 21 UPPSALA, SWEDEN, THE INSTITUTE OF THEORETICAL AND EXPERIMENTAL PHYSICS, MOSCOW, AND THE BURDENKO NEUROSURGICAL INSTITUTE, MOSCOW, USSR.

PERMEABILITY OF THE BLOOD-BRAIN BARRIER IN THE RAT AFTER LOCAL PROTON IRRADIATION

H. LUNDQVIST, K. ROSANDER, M. LOMANOV, V. LUKJASHIN, G. SHIMCHUK, V. ZOLOTOV and E. MINAKOVA

Radiation effects on the adult central nervous system involve more or less marked changes in the vascular system. They are strongly dependent on the radiation dose and field size (BERG & LINDGREN 1963, HAYMARKER 1969).

For whole-head irradiation HOPEWELL et coll. (1970) have suggested that a single dose of 20 Gy gives a reaction on the blood vessels while higher doses have a more direct effect on the parenchyma cells.

The relative importance of parenchymal or endothelial injury is preferably investigated after strictly local irradiation. In experiments performed by LARSSON (1960) with the use of trypan blue and by HASSLER (1966) with peroxidase, vascular abnormalities occurred before pathologic reactions became apparent. Similar results with fluorescein were obtained by VAN DYKE et coll. (1962) and by MINAKOVA & VINOGRADOV (1975).

These and other reports indicate that early changes are difficult to demonstrate with histologic techniques. However, using an adequate substance which normally is not penetrating the brain tissue an early change might be demonstrated by leakage of the substance.

The purpose of the present investigation was to find a method for demonstrating local changes in the blood-brain barrier. A short-lived gamma emitting nuclide, $^{99}Tc^{m}$, with a half-life of 6 hours was used since this nuclide is easily available and is used for the clinical diagnosis of brain tumours. The permeability of the blood-brain barrier system was evaluated after local proton irradiation of the adult rat brain. Repeat examinations were made on the same animal.

Materials and Methods

The experiments were carried out during 2 different sessions, S I and S II, the procedure being mainly identical. However, as rats of different strains were used and the measurements performed with different equipments, the 2 experiments are accounted for separately.

Animals. In the first experimental session, S I, 19 female rats of the Wistar strain were used, and in the second session, S II, 30 female rats of the Sprague-Dawley strain. The weight of the rats at the time of irradiation was 160 to 200 gram.

Irradiation procedure. Irradiations were performed with the 200 MeV biomedical proton beam at ITEP, Moscow (CHOROSHKOV et coll. 1969). The experimental set-up is shown in Fig. 1. A convergent (10 to 25 mrad) beam of circular cross-section passed in air through the collimating system outlined in the figure. The beam was monitored by an induction probe (KLEINBOCK et coll. 1974) immediately after the second collimator (2 in Fig. 1). A

Accepted for publication 13 April 1982.

typical distribution of the flux density after the final collimator appears in Fig. 2.

The absorbed dose was measured with an accuracy of 10 per cent. The dose rate was 50 to 100 Gy/min. Before irradiation the animals were anaesthetized intraperitoneally with Nembutal (5 mg/kg body weight). During irradiation they were fixed in a special holder (MINAKOVA et coll. 1973). Each animal was irradiated once. The beam was directed laterally through the visual cortex. The position of the beam was controlled by roentgenography. Doses and field sizes are given in the Table.

Technique of measurement. During a period of 2 to 58 days after irradiation measurements were repeatedly performed. At each occasion the rat was injected intraperitoneally with 40 MBq $^{99}Tc^{m}$-pertechnetate in 0.5 ml physiologic saline. After 3 to 4 hours the animal was anaesthetized (Nembutal, 5 mg/kg) and the head was scanned by means of a collimated (2 mm slit) NaI detector. The rat was placed supine and the scanning was performed in the antero-posterior direction, either in steps of 1 mm (S I) or by slow continuous movement (0.1 mm/s, S II). The equipment is illustrated in Fig. 3.

Histopathologic examination was performed only in 16 rats in S II. After varying intervals the rats were anaesthetized (Nembutal 7 mg/kg) and cardiac perfusion was performed with 50 ml 5 per cent formalin in physiologic saline. The brain was removed and placed into 10 per cent formalin for one month, embedded in paraplast and cut coronally in sections with a thickness of 5 μ. Staining was performed with haematoxylin-eosin.

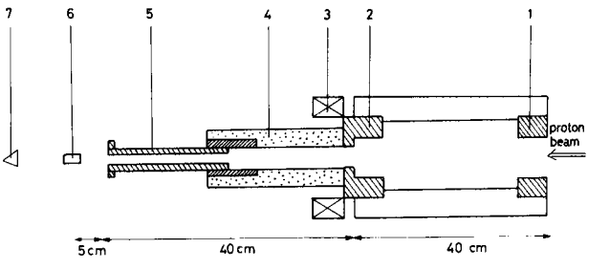

Fig. 1. Experimental set-up for proton irradiation at ITEP, Moscow. 1=first collimator, 2=second collimator, 3=induction probe, 4=collimator holder, 5=final collimator, 6=position of the rat, 7=roentgen tube. The diameters of the aperture of the collimators were 10, 7 and 5 mm in S I and 15, 10 and 7 mm in S II. In S II only collimators 1 and 2 were used to get a beam with a diameter of 7 mm. The first and the second collimator had then an aperture of 10 and 7 mm, respectively. Brass (▨). Ebonite (▩). Steel (■).

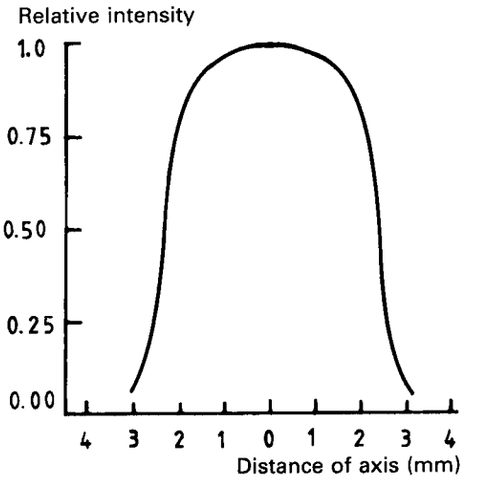

Fig. 2. Flux density distribution of the beam immediately after the final collimator (aperture 5 mm).

Results

All rats increased slightly in weight following the irradiation. They behaved normally during the post-irradiation period. At the experiments, 10 rats died during the anaesthesia. A marked skin reaction within the radiation field occurred; epilation appearing after 13 to 18 days.

Activity distribution of $^{99}Tc^{m}$. The measurement of the uptake in a control rat and in an irradiated rat is shown in Fig. 4. In the control rat the uptake was highest in the salivary glands and in the thyroid regions. In the irradiated rat a high uptake was also recorded about 5 mm anterior to the ears, corresponding to the position of the irradiated field. The height of the peak varied with time after irradiation.

Repeated measurements were performed during

Table

Number of animals in each experimental group

Dose (Gy)	Group S I (n=19)			Group S II (n=30)		
	Beam diameter		Controls	Beam diameter		Controls
	5 mm	7 mm		5 mm	7 mm	
50		5			7	
70		5			6	
100				6		
150	5			6		
Controls			4			5

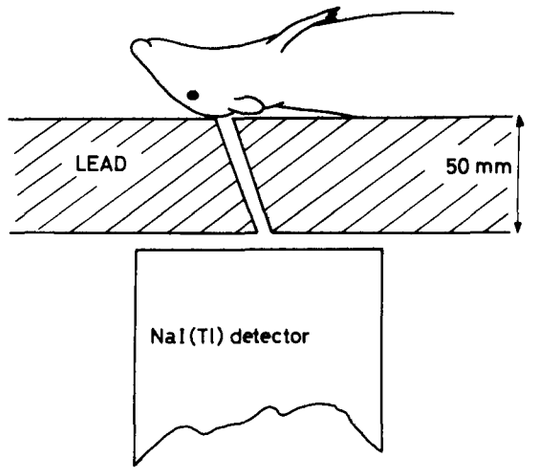

Fig. 3. Experimental equipment for measurement of $^{99}Tc^{m}$ uptake in a rat. Size of detector: 7.5 cm×5 cm (3″×2″).

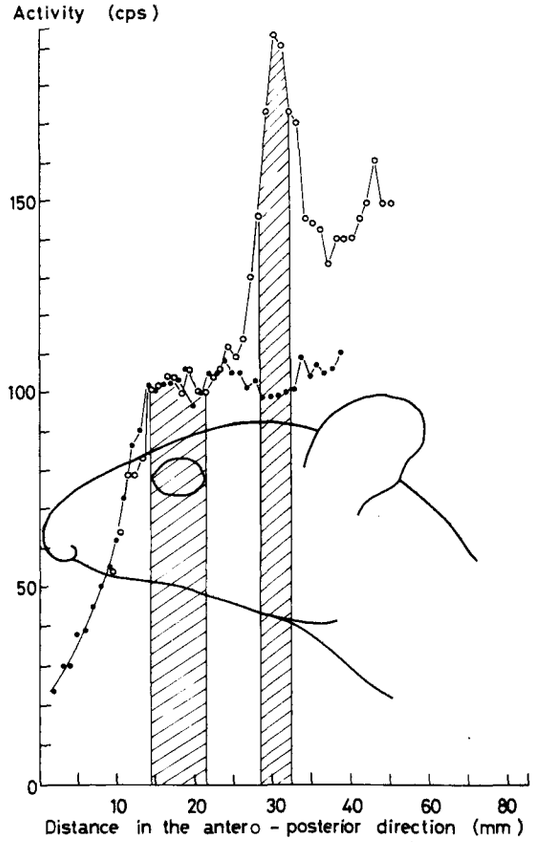

Fig. 4. Distribution of activity in an irradiated rat (○), beam diameter 7 mm, dose 50 Gy, after 37 days and in a control rat (●). The activity is normalized to the same value in the eye area. The position of the eye and of the irradiated region is indicated in the figure.

the post-irradiation period, for some rats up to 10 times.

The measured values of activity were integrated in an area of 7 mm around the eye and of 4 mm around the irradiated area (Fig. 4). The ratio between these values was used as a measure of uptake in the irradiated region. The ratio of uptake in the irradiated animal was then normalized to the corresponding ratio in the control animals (0.48 for S I, 0.64 for S II) and the ratio so formed was taken as an index of $^{99}Tc^{m}$ penetration. This index is shown as a function of post-irradiation time in Figs 5 and 6. From the distributions shown in these figures the following conclusions can be drawn:

(1) For rats irradiated with a beam field diameter of 5 mm a higher uptake occurred after 150 Gy as compared with 100 Gy. Taking the linear trend over days into account the difference in uptake was found statistically significant ($t=3.992$, $p<0.001$). No significant difference according to dose was found for rats irradiated with a beam field diameter of 7 mm.

(2) The time for maximum uptake after irradiation was about 28 days for rats irradiated with a beam field diameter of 7 mm, independent of dose. The corresponding value for a beam field diameter of 5 mm was about 22 days.

(3) The duration of uptake was increased with increased beam field diameter.

Furthermore, the results in S I and S II agreed well.

Histopathology. For each period, only one or two rats were examined.

Beam field diameter 5 mm, dose 100 Gy: After 27 days extensive vascular lesions, minor haemorrhages and local gliosis including erythrophagia were discernible. The neuron injury varied from only slight degeneration to complete cytolysis. After 58 days the lesions were even more marked and circumscribed necrotic areas containing numerous glia scavenger cells predominated. Numerous vessels were heavily injured and in a state of disintegration.

Beam field diameter 5 mm, dose 150 Gy: After 24 days the main findings were necrosis, haemorrhages and glia reactions.

Beam field diameter 7 mm, dose 50 Gy: After 27 days minor haemorrhages, oedema, and areas of glia reactions were discernible. This was also the case after 58 days, but in addition also areas of subtotal necrosis were observed.

Beam field diameter 7 mm, dose 70 Gy: After 16 days minor haemorrhages predominated and on day 26 an additional finding was conspicuous focal accumulation of glia scavenger cells. The structure of the vessels seemed to be normal. On days 39 and 58, besides focal bleeding, also small scattered areas of necrosis were found.

Discussion

Although the histopathologic material is small it gives a rather clear representation of the tissue reactions. Extensive vascular effects occurred in all the rats at the time of maximum uptake of $^{99}Tc^{m}$, for beam diameters 5 and 7 mm occurring on days 22 and 28, respectively. A statistically significant effect was observed with the 5 mm beam. The uptake after 150 Gy was higher than after 100 Gy. It seems that the smaller the beam diameter, the more critical is the choice of dose. The penetration of $^{99}Tc^{m}$ ceases after a certain time irrespective of the occurrence of tissue necrosis.

It has been shown that the increased uptake of $^{99}Tc^{m}$ is related to the absence of intercellular tight junctions (BAR-SELLA et coll. 1979). Further, BENNET et coll. (1976) have observed a close correlation between hexokinase activity and the uptake of $^{99}Tc^{m}$-pertechnetate. In normal irradiated brain tissue it seems reasonable that transport of $^{99}Tc^{m}$-pertechnetate takes place either via injured endothelial cells or via opened tight junctions. MAXWELL & KRUGER (1964) using electron micoscopy found, after alpha irradiation of the rat brain with a beam diameter of 7 mm and doses of 60 or 90 Gy, no indication of the latter suggestion.

When discussing the present results, it has been assumed that the measured activity reflects the amount of $^{99}Tc^{m}$ that has penetrated the blood-brain barrier. Obviously, the measured activity reflects blood volume, blood flow, and extraendothelial penetration. Due to the long time between injection of the nuclide and the scanning it is conceivable that the measured values indicate a penetration including a changed blood flow. It is known that local irradiation (beam diameter 3 mm) decreases capillary function early after irradiation with a dose of 200 Gy (LARSSON 1960). However, this fact does not necessarily imply the same reaction after irradiation with lower doses and beams with diameters of 5 to 7 mm.

In order to verify the assumption of blood-brain barrier penetration, 2 rats irradiated with 150 Gy were perfused after one month with saline and the activity in the removed brain was measured. As compared with the control rats much higher values were obtained in the irradiated region.

In many rats a marked skin reaction occurred. The possibility that $^{99}Tc^{m}$ would diffuse into the injured skin region and interfere with the evaluation of the results was controlled in a preliminary experi-

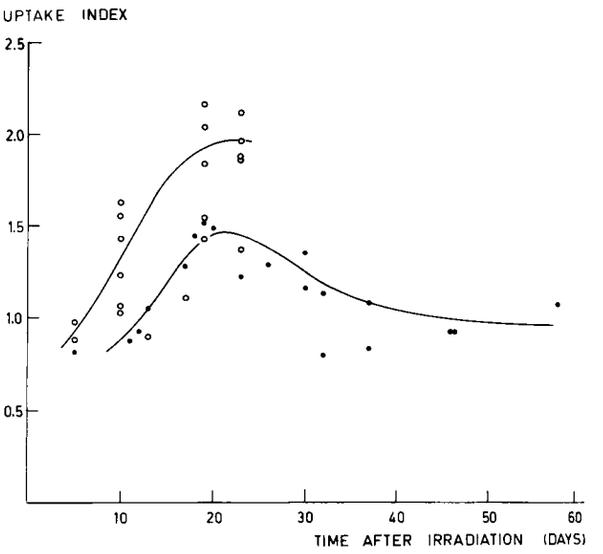

Fig. 5. The uptake of $^{99}Tc^{m}$ as a function of time after irradiation. Beam diameter 5 mm. The error in each point is ±0.03. 100 Gy (●). 150 Gy (○).

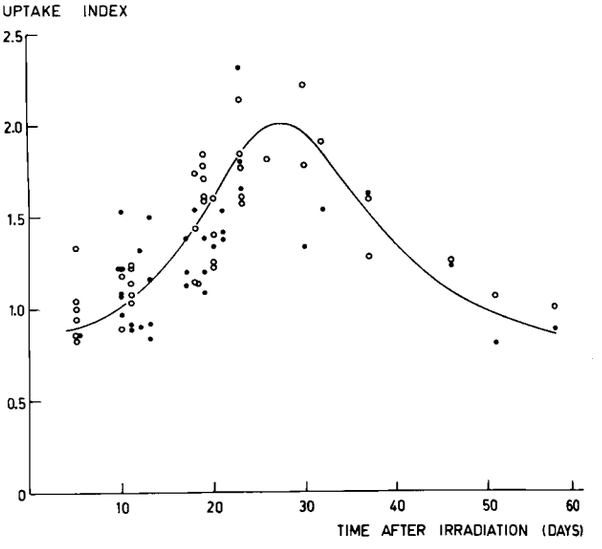

Fig. 6. The uptake of $^{99}Tc^{m}$ as a function of time after irradiation. Beam diameter 7 mm. The error in each point is ±0.03. 50 Gy (●). 70 Gy (○).

ment in which also lower doses were given. No such effect was observed.

It is concluded that the dynamics of the early reaction after irradiation can be investigated experimentally in vivo. The disadvantage of using $^{99}Tc^{m}$ is mainly the high background from the thyroid and the salivary glands. This can be avoided by pharmaceutical treatment.

The uptake of $^{99}Tc^{m}$-pertechnetate has been tested clinically at the Burdenko Institute in Moscow. Seven patients with pituitary adenoma and irradiated with beam fields of 10 to 15 mm and doses of 40 to 100 Gy, were examined during the first 14 days and during 6 to 12 months after irradiation. Furthermore, 2 patients with generalized mammary tumours were examined at 3 to 4 years after irradiation of the normal pituitary in single doses of 120 to 130 Gy. The uptake of $^{99}Tc^{m}$ was measured by gamma camera technique. No activity could be detected. However, other substances than $^{99}Tc^{m}$ could be used to demonstrate vascular reactions after radiation surgery with narrow beams or during fractionated radiation therapy. Differences in sensitivity to radiation could then be detected and the dose adjusted with respect to the treatment time.

SUMMARY

Rats were irradiated laterally through the brain with 200 MeV protons. The beam was of circular cross-section with a diameter of 5 or 7 mm. The doses were 50, 70, 100 and 150 Gy. After irradiation the rats were examined several times by use of injected $^{99}Tc^{m}$-pertechnetate. The uptake of the substance increased to a maximum after 20 to 30 days and then decreased to a normal level. Differences in maximum uptake with respect to dose were significant only for the smaller beam diameter.

ACKNOWLEDGEMENTS

This work has been supported by grants from the Royal Swedish Academy of Engineering Sciences and by grants from the Swedish Medical Research Council. The authors wish to thank Mrs Sonja Andersson for skilful preparations of the histologic sections and Mrs Kerstin Wahlund, Akademiska Sjukhuset, Uppsala, for the accurate preparations of the $^{99}Tc^{m}$-pertechnetate solutions. Valuable discussions with Professor Agnar Nilsson and Professor Börje Larsson are very much appreciated.

Request for reprints: Dr Kerstin Rosander, Department of Physical Biology, Gustaf Werner Institute, P.O. Box 531, S-751 21 Uppsala, Sweden.

REFERENCES

BAR-SELLA P., FRONT D., HARDOFF E., PEYSER E., BOROVICH B. and NIR N.: Ultrastructural basis for different pertechnetate uptake patterns by various human brain tumours. J. Neurol. Neurosurg. Psych. 42 (1979), 924.

BERG N. O. and LINDGREN M.: Relation between field size and tolerance of rabbits brain to roentgen (200 keV) irradiation via slit-shaped field. Acta radiol. Ther. Phys. Biol. 1 (1963), 147.

BENNET M. J., OGILVY K. M., BLAKE G. M., LEWTAS M. and TIMPERLEY W. R.: A study of glycolytic and pentose phosphate shunt enzymes in relationship to the altered permeability in the blood-brain barrier. J. Neurochem. 26 (1976), 1139.

CHOROSHKOV V. S., BARABASH L. Z., GOLDIN L. L., LOMANOV M. F., ONOSSOVSKY K. K. and PLJASHKEVITCH L. N.: Proton beam at the ITEP accelerator for radiation therapy. (In Russian. Summary in English.) Medizinskaja Radiologia 4 (1969), 58.

VAN DYKE D. C., JANSSEN P. and TOBIAS C. A.: Fluorescein as a sensitive, semiquantitative indicator of injury following alpha particle irradiation of the brain. *In:* Response of the nervous system to ionizing radiation, p. 369. Edited by T. J. Haley and S. Snider. Academic Press, New York, London 1962.

HASSLER O.: Microangiographic studies on changes in the cerebral vessels after irradiation. Acta radiol. Ther. Phys. Biol. 4 (1966), 394.

HAYMARKER W.: Effects of ionizing radiation on nervous tissue. *In:* Structure and function of nervous tissue. Vol. 3, p. 441. Edited by G. H. Bourne. Academic Press, New York, London 1969.

HOPEWELL J. W., WRIGHT E. A. and PATH F. C.: The nature of latent cerebral irradiation damage and its modification by hypertension. Brit. J. Radiol. 43 (1970), 161.

KLEINBOCK J. L. and NARINSKY V. M.: A system for flux measuring at the medical and biological proton beam. (In Russian.) Atomnaja Energia 37 (1974), 69.

LARSSON B.: Blood vessel changes following local irradiation of the brain with high energy protons. Acta Soc. Med. Ups. 65 (1960), 61.

MAXWELL D. and KRUGER L.: Electron microscopy of radiation induced laminar lesions in the cerebral cortex of the rat. *In:* Response of the nervous system to ionizing radiation, p. 54. Edited by T. J. Haley and R. S. Snider. Little, Brown and Company, Boston 1964.

MINAKOVA E. I. and VINOGRADOV V. N.: Local lesions after brain irradiation with a narrow beam of protons. (In Russian. Summary in English.) Medizinskaja Radiologia 7 (1975), 33.

— KLEINBOCK J. L., LOMANOV M. F., PAVLONSKY L. M., CHOROSHKOV V. S. and VINOGRADOV V. N.: Irradiation of intracranial structures of small animals by means of a high-energy proton pencil beam. (In Russian. Summary in English.) Medizinskaja Radiologia 2 (1973), 29.