Pergamon

Adv. Space Res. Vol. 14, No. 10, pp. (10)681–(10)684, 1994

0273–1177/94 $7.00 + 0.00

HEAD AND NECK TUMORS AFTER ENERGETIC PROTON IRRADIATION IN RATS

D. Wood,* A. Cox,** K. Hardy,** Y. Salmon** and R. Trotter**

* *Southwest Research Institute, P.O. Drawer 28510, San Antonio, TX 78228, U.S.A.*
** *USAF Armstrong Laboratory, Brooks AFB, TX 78235, U.S.A.*

ABSTRACT

This is a two-year progress report on a life span dose-response study of brain tumor risk at moderate to high doses of energetic protons. It was initiated because a joint NASA/USAF life span study of rhesus monkeys that were irradiated with 55-MeV protons (average surface dose, 3.5 Gy) indicated that the incidence of brain tumors per unit surface absorbed dose was over 19 times that of the human tinea capitis patients whose heads were exposed to 100 kv x-rays. Examination of those rats that died in the two-year interval after irradiation of the head revealed a linear dose-response for total head and neck tumor incidence in the dose range of 0-8.5 Gy. The exposed rats had a greater incidence of pituitary chromophobe adenomas, epithelial and mesothelial cell tumors than the unexposed controls but the excessive occurrence of malignant gliomas that was observed in the monkeys was absent in the rats. The estimated dose required to double the number of all types of head and neck tumors was 5.2 Gy. The highest dose, 18 Gy, resulted in high mortality due to obstructive squamous metaplasia at less than 50 weeks, prompting a new study of the relative bological effectiveness of high energy protons in producing this lesion.

INTRODUCTION

During the period 1964-1969, the U. S. Air Force School of Medicine (USAFSAM), with the support of the National Aeronautics and Space Administration (NASA), conducted experiments to ascertain the acute effects of proton irradiation in rhesus monkeys. Monkeys of both sexes were exposed to one of several dose and energy combinations to envelop the known spectrum of proton radiations in space. The energies chosen were 32, 55, 138, 400 and 2300 MeV. The life span study involved 301 subjects of the original experiments and a group of age matched controls that were continuously observed to determine the long term effects, especially cancer, cataracts and life shortening /1, 2/. Among the cancers occurring in the monkeys, the most prevalent single type was glioblastoma multiforme, a malignant brain tumor that affected one female and eight males in a group of 72 animals exposed to 55-MeV protons /3/. Protons of this energy will transfer all of their kinetic energy within the first 2.5 cm of tissue, resulting in an unequal (Bragg peak) dose distribution in the head of a monkey /4/. The dose range for the affected monkeys was 4 to 8 Gy, but some areas of the brain could have received up to three times the measured surface dose /5/. The first brain tumor appeared 18 months after irradiation, while the last occurred 20 years later.

The present study was undertaken to aid in the interpretation of the primate data by utilizing a large population of Fischer-344 rats to establish the dose-response relationship between Bragg peak energetic protons and brain tumors. Fischer rats are useful in carcinogenesis studies because of their relatively low spontaneous solid tumor rate and a positive response to several known carcinogens. In reviewing brain tumor incidence in control groups of Fischer rats used in 2-year carcinogenesis studies, Solleveld et al., reported that the overall incidence was 1.04% among 5450 subjects and that it never exceeded 4% in any single control group of 50 animals /6/. Because malignant gliomas occur with a predictable frequency in humans, establishment of a dose-response curve for brain tumor induction by proton

irradiation will permit estimation of cancer risk subsequent to space missions and help establish the probability of causation for those tumors that actually do occur in crew members later in life.

METHODOLOGY

Eleven hundred male Fischer-344 rats*, aged 70 days, were delivered directly to the Harvard Cyclotron Laboratory, Cambridge, MA, 24 hours prior to the scheduled exposures. The test animals were divided into five dose groups of 200 animals each, with an additional 100 animals retained for quality control monitoring at scheduled intervals. The dose groups were zero (sham), 2, 4, 8.5 and 18 Gy. Unanesthetized animals were irradiated in groups of eight in a circular restraining device, positioned and shielded so that only the heads were exposed to the proton beam. The 138-MeV beam was attenuated by Lucite filters to produce an expanded Bragg peak and a uniform dose distribution across the area of the entire brain at a rate of approximately 1.25 Gy/min. Dosimetry was verified by exposing rat cadavers containing implanted thermoluminescent dosimeters.

At 923 days after irradiation, with less than 2% of the subjects alive, all remaining animals were killed and examined. Every subject in the study received a complete post-mortem examination including serial sections of the brain for histological verification of tumor occurrence and type.

RESULTS

Survival

Survival data for all experimental groups for the duration of the study are given in Table 1.

Table 1 Survival Data for Male F-344 Rats after Head-Only Proton Irradiation

Dose	No. Subjects*	Mean Survival (Weeks)	Median Survival (Weeks)	R_2**	R_T**
0.0 Gy	223	104.4	110.7	3933	23270
2 0 Gy	198	97.3	101.8	7379	20060
4.0 Gy	162	96.8	99.9	5749	15671
8.5 Gy	198	90.0	91.3	9361	17818
18.0 Gy	143	39.0	33.6	5119	5582

* Different from the total number of animals exposed because accidental mishandling by the commercial carrier disqualified some subjects.
** R_2, weeks at risk, two-year mortality. R_T, total weeks at risk.

The median survival time in the controls was 845 days after birth. This is consistent with control data in Fischer rats published by the National Institute of Environmental Health Sciences National Toxicology Program (NTP) /7/. The high early mortality in the 18-Gy group was primarily attributable to respiratory complications associated with squamous metaplasia of the nasopharynx. This condition was infrequently seen in lower dose groups, where it was never identified as a factor in the animal's death. Determination of the probable causes of death and the tabulation of the pathological findings other than cancers of the head and neck is still in progress and will be published in a later report.

Incidence of Head and Neck Tumors

At the time of this report, complete data on the incidence of head and neck tumors was available for those subjects that were examined during the two-year post irradiation period. This conforms with the standard two-year rodent carcinogenesis study established by the NTP, but does not include tumor data from animals examined after the two-year point. Analysis of the life span data is still in progress. For

*CDF® (F-344)/CrlBR, Charles River Laboratories.

the combination of all types of head and neck tumors, there is a dose-ordered incidence that shows a high degree of linearity if data from the 18-Gy group are excluded from the regression (Figure 1).

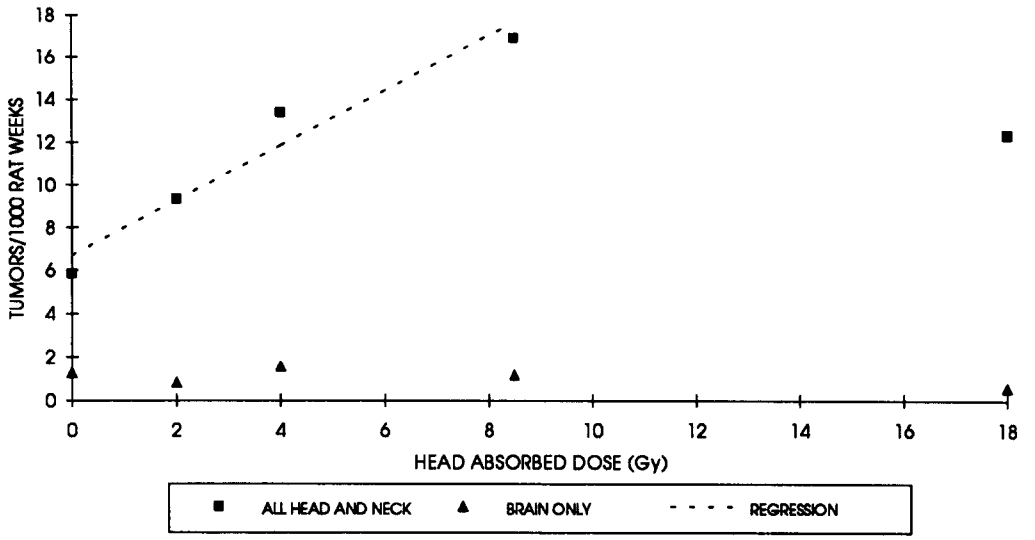

Fig. 1. Two-year incidence of head and neck tumors in five dose groups.

The slope of the regression line predicts that the dose required to double the number of brain tumors would be 5.2 Gy. When the tumors are grouped by cell type, significant increases in the incidence of pituitary chromophobe adenomas, epithelial cell and mesothelial cell tumors can be identified by χ^2 testing (Figure 2).

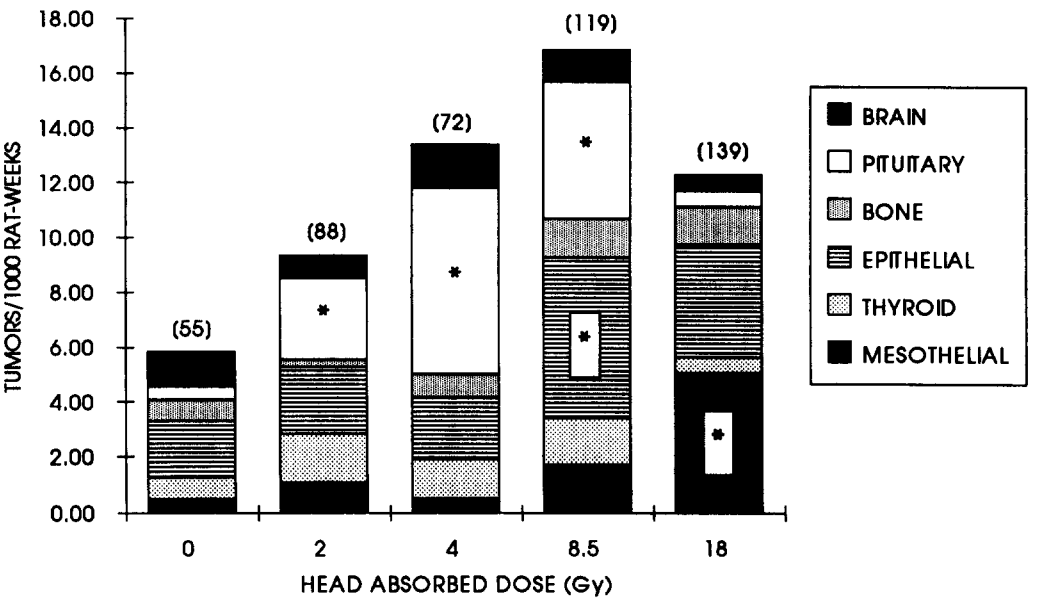

Fig. 2. Two-year incidence of head and neck tumors by primary cell type. Figures in parentheses are the number of animals examined at each dose. * $p < .05$ by χ^2.

DISCUSSION AND CONCLUSIONS

Data from those subjects that died in the two-year interval after irradiation or were sacrificed because of terminal illness indicated that the incidence of total head and neck tumors was significantly greater than the controls in every exposure group. Pituitary adenomas accounted for the greater part of this increase in the 2 and 4-Gy groups, while epithelial and mesothelial cell tumors were more prevalent in the 8.5 and 18-Gy groups. There was no relationship between the exposure to energetic protons and the occurrence of malignant gliomas of the type that was seen in proton-irradiated monkeys. It should be noted, however, that almost 60% of the non-irradiated rats lived longer than two years. It is possible that the life span incidence of brain tumors in the control population may be less than the observed 9% in those animals dying before the two-year point. That incidence seems abnormally high in view of the overall 1.04% (4% maximum) reported in two-year studies by the NTP /7/. The absence of any significant increase of malignant glial cell tumors suggests that the two-year study may be too short to elucidate any change.

Most of the tumors were discovered on histological examination and were not identified as a primary cause of death. Aging Fischer rats have a high incidence of fatal mononuclear cell leukemia (MCL), which accounted for many of the deaths in all groups including controls. The life span results will include an analysis of the relationship of MCL to the dose of radiation, but none was evident at the two-year point.

The high mortality due to airway obstruction by squamous metaplasia in the 18-Gy group undoubtedly precluded the expression of many tumors with long latent periods. This lesion rarely occurs spontaneously, but is commonly seen as a response to chronically inhaled irritants. It is regarded as pre-cancerous and may develop into squamous cell carcinoma /8/. The high incidence of squamous metaplasia was not anticipated in the experimental design and an additional study, using identical doses of ^{60}Co γ-radiation, is now in progress to estimate the relative biological effectiveness of Bragg peak protons in inducing this lesion.

REFERENCES

1. D.H. Wood, Long-term mortality and cancer risk in irradiated rhesus monkeys, *Radiat. Res.* 126, 132-140 (1991).

2. J.T. Lett, A.B. Cox, D.S. Bergtold, A.C. Lee and J.E. Pickering, Cataractic potential of ionizing radiation in animal models that simulate man, *Adv. Space Res.* 6(11), 295-303 (1986).

3. D.H. Wood, M.G. Yochmowitz, K.A. Hardy and Y.L. Salmon, Occurrence of brain tumors in rhesus monkeys exposed to 55-MeV protons. *Adv. Space Res.*,6(11):275-283 (1986).

4. I.R. Lindsay, G.V. Dalrymple, J.J. Ghidoni, J.C. Mitchell and I.L. Morgan, Some effects of 55-MeV protons on primates, *Radiat. Res.*, 28:446-464 (1966).

5. D.D. Leavitt, Analysis of primate head irradiation with 55-MeV protons, *Radiat. Res.*, 126, 127-131 (1991).

6. H.A. Solleveld, S.H. Bigner, G.A. Boorman and D.D. Bigner, Neoplasms of the central nervous system, in: *Atlas of Tumor Pathology of the Fischer Rat*, ed. S.F. Stinson, H.M. Schuller and G.K. Resnik, CRC Press, Boca Raton FL (1990), p. 486.

7. H.A. Solleveld, J.K. Haseman and E.E. McConnell, Natural history of body weight gain, survival and neoplasia in the F344 rat, *J. Natl. Cancer Inst.*, 72: 929-940 (1984).

8. G.A. Boorman, K.T. Morgan and L.C. Uriah, Chapter 20. Nose, Larynx, and Trachea, in *Pathology of the Fischer Rat*, ed. G.A. Boorman, S.L. Eustis, M.R. Elwell, C.A. Montgomery, Jr. and W.F. MacKenzie, Academic Press, Inc., San Diego CA (1990), p. 329.