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NEUTRON ISSUES IN THE JANUS MOUSE PROGRAM*

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INTRODUCTION

In a description of the efforts made to predict the late effects of radiation exposure for the 30 years prior to 1969, Grahn (1) suggested that "there is really nothing new or untried in this kind of activity, although the emphasis placed upon one or another procedure has varied." His comment should not be interpreted as a criticism, but rather as an indication of the complexity of the problem. One of the most difficult aspects in assessing radiation risk is the relationship between dose and time (2). An enumeration of key factors in the dose-time relationship would have to include dose rate, total dose, protraction period, number of fractions, and the interval between fractions (3).

Extensive research on experimental animal populations (principally, mice and dogs) exposed to radiation has demonstrated that the predominant cause of excess mortality is cancer, especially at the occupational exposure levels of interest in humans. The estimation of risk for radiation - induced cancer in man has been largely limited to persons exposed occupationally or therapeutically and to survivors in Hiroshima and Nagasaki. Current estimates of cancer risk derived from human data rely on extrapolations across time because data on the lifetime cancer experience of irradiated human populations simply does not exist (4). With few exceptions, documented carcinogenic effects of radiation in humans have been restricted to populations exposed to relatively high doses and dose rates (4). Issues of dosimetry, pattern and level of exposure, and radiation quality have complicated the data analysis in studies of humans chronically exposed to radiation. Therefore, animal studies are needed to provide the basic expectations necessary for risk assessment (i.e., which neoplasms are likely to arise, when they occur, and how many to expect) and to design and interpret studies of mechanisms.

Nuclear accidents and exposure of workers in nuclear facilities have emphasized the need for better knowledge about the human response to radiation, and regulatory agencies have a specific need for information in order to establish standards. The latest revision of the dosimetry for the A-bomb survivors has left virtually no human data available for evaluating the effects of direct exposure to neutrons (5). Further, the data for the single brief exposures to gamma rays experienced by the bomb survivors can never allow us to address the critical issues of the dose-time relationship for low-LET radiations. Because relevant long-term, whole-animal experiments are limited in number, expensive, and time consuming, there is a critical need to improve upon old methods or to develop new methods for extracting information from existing animal databases.

Over the last 25 years, the JANUS program in the Biological and Medical Research Division at Argonne National Laboratory (ANL) has compiled a database on the response of both sexes of an F_1 hybrid mouse, the $B6CF_1$ (C57BL/6 x BALB/c), to external whole-body irradiation by ^{60}Co γ-rays and fission neutrons. Three basic patterns of exposure for both neutrons and γ-rays have been investigated: single exposures, 24 equal once-weekly exposures, and 60 equal once-weekly exposures. All irradiations were terminated at predetermined total doses, with dose calculated in centigrays at the midline of the mouse.

Three endpoints will be discussed in this paper: (i) life shortening, (ii) a point estimate for cumulative mortality, and (iii) the hazard function. Life shortening is used as an analysis endpoint because it summarizes, in a single index, the integrated effect of all injuries accumulated by an organism. Histopathological analyses of the mice used in the ANL studies have indicated that 85% of the deaths were caused by neoplasms. Connective tissue tumors were the dominant

tumor in the $B6CF_1$ mouse, with tumors of lymphoreticular origin accounting for approximately 80% of this class. The latter two endpoints will therefore be used to describe the life table experience of mice dying from the lymphoreticular class of tumors. Dose-response models will be applied to the three endponts in order to describe the response function for neutron exposures, evaluate the effect of dose range and pattern of exposure on the response function for neutrons, and provide a set of neutron relative biological effectiveness (RBE) values for the ANL database.

METHODS

Mean aftersurvival (MAS) for all causes of death was used as the response variable in the life-shortening analyses. Dose-response models were generated by weighted regression (6) using dose or functions of dose as predictor variables and the inverse variance of MAS as a weighting device. For linear or linear-quadratic models, the slope coefficient for the linear term (with sign reversed) was interpreted as a life-shortening coefficient. The models were fitted to the neutron data at a series of progressively increasing dose ranges, subsequently called the "truncated" data because the responses above each specified dose range were ignored. Identical models were also fitted to the γ-ray data, but for these analyses the entire dose range was included in the analysis. Separate models were determined for each sex, radiation quality and pattern of exposure. All equations were constrained through the concurrent control values.

Cumulative mortality (7) from lymphoreticular tumors was calculated (8) at intervals of 200 days after initial exposure for every dose point in the database. The mortality estimate for the interval from 800 to 999 days was then used as the response variable in dose-responsive models as described for the life-shortening endpoint. Visually, the values for the response variable are

the intersections of the mortality curves (Fig. 1) with a line drawn perpendicular to the x-axis (time) at 900 days, which were then regressed on dose (see inset).

The final endpoint is the age-specific central death rate (9) or mid-interval estimate of the hazard function (10) for deaths from lymphoreticular tumors. Age intervals after initial exposure were chosen to be 50 days. In these analyses, the hazard function is the probability of death from a lymphoreticular tumor during a very small time interval, assuming that the mouse has survived to the beginning of the interval. In other words, it measures the risk of death per unit time during the aging process of a mouse (8). The form of the basic model (11) is given by

$$\mu(t;d) = \lambda(t)[1+\phi(d)\omega(t)] \qquad (1)$$

where $\lambda(t)$ is the hazard function at time t for a control population, $\phi(d)$ is a linear or linear-quadratic function of dose (d), and $\omega(t)$ is an exponential (i.e., loglinear) function of time. Using this framework, the death rate for a particular dose group at time t, $\mu(t;d)$, is a multiple of the death rate observed in the control population. As in the prior analyses, separate models were fitted for each sex, radiation quality, and exposure pattern. All models were constrained through the hazard function for a pooled historical control. Coefficients for this model were determined using iteratively reweighted least squares by the AMFIT (11) software used to generate risk estimates in the BEIR V report (4).

Relative biological effectiveness values (neutron vs. γ-rays) for the life-shortening and cumulative mortality analyses were estimated by the ratio of linear slope coefficients (12) within a pattern of exposure, even when derived from a linear-quadratic model. RBE values and protraction effectiveness factors (PEF) were determined for the hazard function endpoint by calculating isoeffect contours. Isoeffect contours were determined by equating the excess risk

terms, $\phi(d)\omega(t)$, for either radiation qualities (to give RBE values) or patterns of exposure (to give PEF values) and solving the equation for the ratio of doses that produced an identical biological effect.

RESULTS

A variety of models were fitted to the data for life shortening (13), but only the simple linear and linear-quadratic models will be discussed in this paper. Curvature in the response of females or males (Table 1) to neutron exposure could not be detected below 160 cGy. Although the response to fractionated exposures (24 and 60 once-weekly) began to separate from the response to single exposures by 40 Cgy, significant augmentation with dose protraction could not be statistically detected until the 80 Cgy dose point was included in the analysis. Prior to the emergence of neutron-induced augmentation, the pooled estimates of life shortening (i.e., days lost per Cgy) for either sex became progressively larger as the modeled dose range was restricted (truncated) to lower total doses (Table 1). The response to gamma rays (Table 2) was invariably linear and decreased as the exposure was protracted. RBE values (13) ranged from 6 to 43, depending on the dose range used for neutron exposure and the exposure pattern selected as the γ-ray baseline. The neutron dose range had only a small effect on estimates of RBE_m, and the neutron augmentation effect had no influence on RBE_m for the life-shortening endpoint.

The dose-response equations for lymphoreticular tissue tumors that caused or contributed to death were, with only two exceptions, linear for both sexes and both radiation qualities (Table 3). Relative to single neutron exposures, there was a significant increase in the risk of death per Cgy for males given 24 once-weekly exposures and females receiving 60 once-weekly

exposures. It must be said, however, that no consistent augmentation from neutron exposure was apparent for cumulative mortality within the interval of 800 to 999 days after first exposure. The reduced effectiveness of γ-ray exposure with dose protraction observed for the life-shortening endpoint occurred only in males for this neoplastic endpoint. RBE values, ranging from 3 to 16, were consistent with those observed for life shortening.

A somewhat different picture emerges for lymphoreticular tumors when the time domain is incorporated into the modeling process via the hazard function. Equating the estimated equation for single exposures with each protracted/fractionated exposure and isolating the ratio of doses (d_S/d_P) provides an estimate of the protraction effectiveness factor (Table 4). The PEF equals one if there is no effect, is greater than one if protracting the dose increases (augments) the effect, and is less than one if the effectiveness decreases with dose protraction. For either sex exposed to neutrons, the effect from protracting the neutron dose did not depend on the dose delivered. In general, augmentation diminished with time since first exposure and was nonexistent by 900 days (i.e., the time interval used for the cumulative mortality analysis). The neutron augmentation effect was greater in females but appeared to diminish more rapidly with time than in males. When PEF values were generated for γ-ray exposures (Table 4), the disparity between single exposures and protracted/fractionated exposures increased with time.

RBE values were generated in a numerically similar way, except that the radiation qualities were matched by pattern of exposure. Except for males receiving 60 once-weekly exposures and females receiving single exposures, RBE values remained relatively constant through time (Table 5). If attention is restricted to 900 days post exposure, the RBE values from the hazard function

analysis are consistent with those observed for lymphoreticular tumors in the cumulative mortality analysis.

DISCUSSION

The quote used to open this paper was selected to emphasize the importance of procedure in risk analysis. The common theme of the endpoints described in this paper is that they are all derived from a life table. An emphasis on the use of life table statistics (1) has been a tradition in the JANUS program (14) and has its antecedents in the pioneering work of George Sacher (15) at Argonne. This paper simply represents another step in the evolution of life-table-based methods used in the analysis of data from the JANUS program. The life table regression procedure of Cox (16) and its later generalizations (11,17,18) now permit the critical time domain to be explicitly incorporated into the models used for radiation risk assessment.

The existence or importance of an augmented effect with dose protraction of neutrons has been an issue in radiation biology for some time. In his summary of the 1963 ICRP-ICRU report (12), Sinclair (19) stated the panel's view that "even if higher RBE's are found at lower doses in the future, this may be because of lesser effects for the low-LET radiation rather than increased effects for high-LET." In a similar vein, Upton (20) suggested that "the effectiveness of high-LET radiation is less dependent on dose rate than is that of low-LET for many, if not most, biological effects in mammals." The presence of augmentation with protraction of the neutron dose depended on the neoplastic endpoint (21) in analyses of the mouse data at Oak Ridge National Laboratory. Neutron-induced augmentation has, however, been documented for

genetic injury (22), transformation in mammalian cells (23), life shortening (13), and tumor mortality (24).

For any given age interval, cumulative mortality reflects all prior mortality experienced by a population, whereas the hazard function represents only the mortality within the specified age interval. Life shortening is an even more extreme summary measure in that it reflects the mortality experienced across the entire time domain. Any endpoint that summarizes mortality across the time domain will lose information. A contrast of the cumulative mortality and hazard analyses for lymphoreticular tumors in the JANUS database demonstrates the effect this may have on data interpretation. The cumulative mortality analysis provided no clear indication of neutron augmentation within the 800- to 999-day age interval. The hazard analysis, when evaluated at 900 days, led to the same conclusion. However, by including the time domain in the model, the hazard analyses suggest that an augmentation phenomenon does exist but its detection is strongly time-dependent. Revisiting databases with relatively standardized time-dependent models may resolve some controversies where prior analyses, based on summary statistics, have led to conflicting interpretations.

Another area of interpretive interest in the JANUS program is the estimation of RBE values. Here again, when attention is restricted to 900 days after exposure, the RBE values resulting from the hazard analyses (Table 5) are in basic agreement with those derived for cumulative mortality (Table 3). The nearly constant RBE values for all exposure patterns and both sexes occur because neutron augmentation diminishes with time and the decreased effectiveness of protracted exposure to γ-rays increases with time. The two effects are of comparable magnitude and cancel each other out. RBE values for the lymphoreticular tumors

are in the recommended neighborhood of 20. The protraction effectiveness factors (Table 4) for γ-rays can be used as multiples to convert the RBE values reported for protracted exposure to neutrons to those that would result if single exposure to γ-rays were selected as the baseline. The comparable results presented for the cumulative mortality and hazard analyses occurred because there were no time-by-dose interactions in the JANUS database for the lymphoreticular endpoint. If such interactions had been present, interpretive differences for the two endpoints would have resulted.

In discussing the quality factor, Fry (25) indicated that "RBE's vary with dose, dose rate, and fractionation, and are tissue-dependent." The results of the hazard analyses would suggest that time dependence should be added to the list of factors influencing the magnitude of RBE values. The practical effect of this long list of influencing factors is that no single RBE value can adequately represent even a single biological endpoint, let alone the complex array of neoplastic events characteristic of the response to neutron exposure. Coupling the inherent variability of RBE values with the continuing controversy over the appropriate exposure pattern (single vs. protracted) and the specific radiation (x-ray vs. γ-ray) used to represent the low-LET baseline forces one to question the usefulness of the RBE concept in radiation protection. Given the current lack of practical alternatives to RBE values, there is a need to be sensitive to the problems inherent in their use. These issues will become even more critical with the increased need to derive credible risk estimates for humans from the animal data.

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Fig. 1. Intersections of the cumulative mortality curves at 900 days after initial exposure used for dose-response modeling (insert).