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Early and late effects of fractionated irradiation of the thorax of WAG/Rij rats

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While knowledge about early effects of radiation on normal tissues has increased considerably, due to a great number of clinical and animal investigations, very few data are available on very late effects, i.e., complications that will arise several years after treatment. One of these late complications of radiation treatment is lung fibrosis, a very slowly progressing lesion capable of completely disabling the patient several years after radiation therapy.

Unconventional fractionation regimens, e.g. multiple fractions per day, are being increasingly applied in radiotherapy. When tolerance doses are to be calculated using isoeffect models like the LQ model presented by Barendsen (1982), it is important to know how late responding tissues react with changes in fractionation schemes, since the effect on early and late responding tissues might be different and late effects might be dose-limiting.

The object of our studies is to determine the halftime for repair of sublethal damage (important when fractions are given at short intervals) and the α/β ratio, using the formation of radiation fibrosis in rat lung as the endpoint.

Materials and methods

Female rats of the WAG/Rij strain were used at an age of 12 weeks at the beginning of treatment.

Irradiations were performed with 300 kV X-rays at a dose rate of 0.9 Gy min^{-1}. The animals were irradiated to the whole thorax bilaterally (Lopes-Cardozo *et al.*, 1985). Hence, the forelegs, the heart and part of the spinal cord were included in the treatment field. The remainder of the body was shielded by 5 mm lead.

Anaesthesia was given with an Ethrane/air mixture (Lopes-Cardozo *et al.*, 1985). Very fast induction and recovery of the anaesthesia were obtained, allowing the animals to eat and drink between irradiations even when irradiated at 1 h intervals.

Irradiations were given in 1 to 16 fractions at 6 h intervals. This time interval was chosen to limit the overall treatment time with the 8 and 16 fraction treatments in order to avoid the influence of slow repair (Field *et al.*, 1976). For each fraction number, a specific dose was given at time intervals of 1, 2, 4 and 6 h, to get an indication of the rate of repair of sublethal damage. The treatment regimens are listed in Table I.

Table I Doses and intervals used in single and fractionated thoracic irradiations

Number of fractions	*Range of fractional doses (Gy) given at 6 h intervals*	*Fractional doses (Gy) given at 1, 2, 4 and 6 h intervals*
1	5.25–14.00	—
2	3.76– 9.00	5.50; 7.25
4	2.95– 5.50	4.23
8	1.60– 2.95	2.28
16	0.90– 1.60	1.08

Follow up of the animals was done by measuring breathing rate and tidal and minute volume at regular time intervals. A pneumotachograph (Gould, Bilthoven, The Netherlands) was used equipped with a Fleisch Flow Transducer (type 3 × 0). This was connected to a plethysmografic tube (Coggins *et al.*, 1981) in which the rats were placed. A full description of the technique will be given in a separate communication.

When the animals died or were killed as they deteriorated, the lungs were inflated with buffered formalin and the thoracic organs were removed *en bloc* and fixed in formalin. The right lung lobe was removed for determination of hydroxyproline content (results will be reported elsewhere), and the remainder of the lung and the heart were histologically examined, using 5 μm thick hematoxylin-phloxine-safran (HPS) stained slides. Additional collagen staining by Azan or Van Gieson were applied when indicated.

Results

The fractional doses were selected using an upper limit for the total dose in each experimental group

in the order of the LD_{50} value for radiation pneumonitis for mice and rats based on data in the literature, i.e., ~14 Gy for single fractions. No mortality was expected before radiation pneumonitis would have developed, i.e., not until about 12 weeks post-irradiation (Travis, 1980; Travis *et al.*, 1980). However, in the period between 4 and 12 weeks after irradiation, rats from the highest dose groups treated with 1, 2, 4 and 8 fractions, died. These deaths were preceded by elevated breathing rates (visually obtained, since the pneumotachograph setup was not completed until 16–20 weeks after the start of the experiments). There was a clear relationship between radiation dose and time of death. Figure 1 illustrates this for animals irradiated with single doses. A similar dose-effect relationship was found after 2, 4 and 8 fractions.

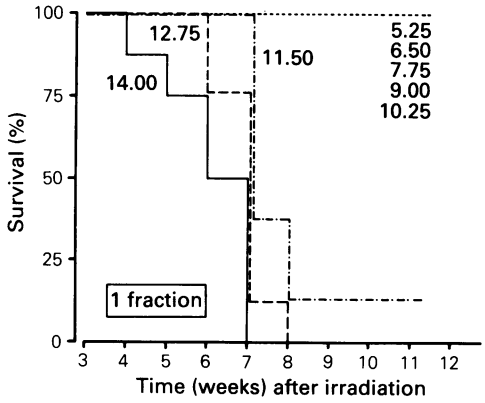

Figure 1 Mortality pattern after single doses of X-rays with doses as indicated.

Fractionation had a sparing effect on early mortality (Figure 2). LD_{50} values after 1, 2 and 4 fractions were 11.3, 14.4 and 19.7 Gy, respectively.

After 12 weeks, only 2 more animals died (at 21 and 33 weeks, respectively, but histologically without pneumonitis or lung fibrosis). No changes in breathing rate and tidal volume could be detected for up to 46 weeks post-irradiation. At 52 weeks, animals in some dose groups showed a small but significant increase in breathing rate and decrease in tidal volume, which persisted at later times. No dose-response relationship could be observed, however.

Discussion

The sequence of histological and functional changes in mouse lungs has been published by Travis and

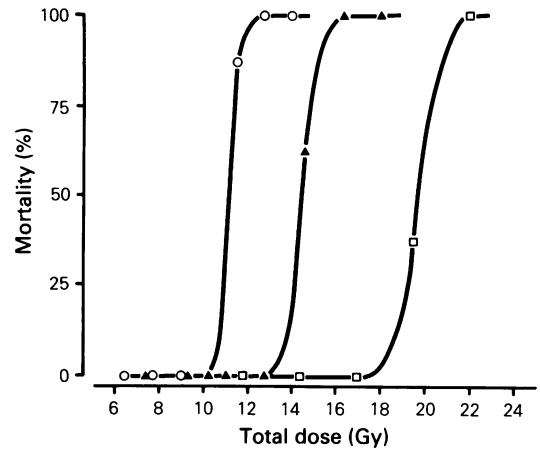

Figure 2 Dose-mortality curves obtained 12 weeks after 1 (○), 2 (▲) and 4 (□) dose fractions of X-rays given to the thorax.

co-workers (Travis, 1980; Travis *et al.*, 1980). As in man, radiation pneumonitis in mice occurs in the period of about 12 to 28 weeks post treatment. In this period an increased breathing rate was observed and pneumonitis could be demonstrated histologically. Between 28 and 36 weeks breathing rate decreased again when the animals recovered from pneumonitis. After 36 weeks, a slow but steady rise in breathing rate was observed due to an increase in the amount of collagen deposited in the lung tissue (radiation fibrosis).

In view of the time of occurrence of radiation pneumonitis in man and rodents, the early deaths of our WAG/Rij rats were quite unexpected. The cause of death in this early mortality phase is not completely clear. Pneumonitis could be demonstrated as early as 6 weeks after irradiation. In several cases it was severe enough to cause the death of the animal.

Since after this early mortality, only some incidental deaths occurred without histological signs of pneumonitis or increased breathing rate, it might be concluded that radiation pneumonitis occurs unusually early in WAG/Rij rats. These results are comparable to those reported by Lopes-Cardozo (1985) in Bn/BiRij rats.

In a number of the animals which died early, only minimal signs of pneumonitis could be found histologically. Another possible cause of death is cardiac failure due to increased pulmonary arterial hypertension. Right ventricular hypertrophy was regularly observed. We also observed in a number of cases a massive accumulation of pleural fluid, sometimes of over 10 ml, which is almost the entire volume of the thorax. The time sequence of fluid accumulation, however, is unknown. The time

sequence of fluid accumulation, however, is unknown. Thus the possibility that increased breathing rate already existed before the occurrence of massive fluid accumulation cannot be excluded.

In a separate experiment 3 animals were killed 7 weeks after receiving 12.75 Gy to the thorax (a dose that resulted in 100% mortality within 8 weeks in the first experiment). The breathing rate of these animals was normal and they appeared healthy. No pleural fluid was found at autopsy, which is in favour of a fast accumulation shortly before death.

Whatever the exact causes of death were, the radiobiological parameters that could be derived from the data are comparable to those obtained by other authors for radiation pneumonitis. From the LD_{50} data derived from the mortality curves in Figure 2, an α/β ratio of 3.8 Gy could be derived using regression analysis on the inverse total dose vs fraction size (Barendsen, 1982). This α/β ratio is well within the range of ratios for pneumonitis as listed by Travis *et al.* (1983) for mice. With the values of the LD_{50} at 12 weeks, the percentage dose recovered of the total dose, F_R, and of the fractional dose, F_{rec}, were calculated, according to Travis *et al.* (1983) for the 4-fraction data. Values for F_R and F_{rec} are 43% and 58%, respectively. The values obtained by Travis with the breathing rate assay at 16 weeks were 42% and 54%, respectively.

From the preliminary data obtained with varying time intervals the rate of repair appeared to be dose related and with the 4, 8 and 16 fractions employed, to be virtually complete within 6 h. This is in reasonable agreement with the $T_{\frac{1}{2}}$ of 1.5 h as calculated by Thames *et al.* (1984) for LD_{50} in mice due to pneumonitis.

No changes in breathing rate could be detected in the WAG/Rij rats up to 46 weeks. This is in contrast to the observations of Travis *et al.* (1980) that lung fibrosis could be detected by a progressive increase in breathing rate beyond about 36 weeks post-irradiation. Even at 52 weeks there was not a sufficient response so that dose-effect relationships could be determined. Perhaps the rat has a large reserve capacity of the lung, the utilization of which initially masks the development of fibrosis. Rats surviving after 78 weeks will be killed and fibrosis will be determined biochemically and histologically.

Experiments are now in progress to determine the breathing rate of control and irradiated rats while breathing air with a lower oxygen tension, to force hyperventilation and to increase the sensitivity of the breathing rate assay for small changes in oxygen diffusing capacity. When changes can be detected in this way, rats with the clearest changes will be killed for histological determination of lung fibrosis and for hydroxyproline content.

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