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Late Renal Damage After Total Body Irradiation and Bone Marrow Transplantation in a Mouse Model: Effect of Radiation Fractionation

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In response to the accumulating evidence that renal damage is now becoming an important late complication after total body irradiation (TBI) and bone marrow transplantation (BMT), we have tested the effect of fractionated and hyperfractionated TBI on late kidney damage in a mouse model. TBI was given as fractionated (three fractions in 3 days, Fx 3), or hyperfractionated (nine fractions in 3 days, Fx 9) treatment. Kidney damage was evaluated using [^{51}Cr]EDTA residual activity, blood urea nitrogen (BUN) and percentage haematocrit (%Hct) as end-points. We were able to detect progressive renal damage with no evidence of recovery or plateau in the Fx 3 and Fx 9 schedules. The time latency before the expression of renal damage was dependent on both total dose and end-point and it was shorter the higher the dose. [^{51}Cr]EDTA detected renal damage at the same doses as BUN but earlier in time whereas %Hct detected renal damage at doses lower than both BUN and [^{51}Cr]EDTA. Reducing the dose per fraction spared the kidney from TBI damage. The dose–response curves for renal damage (using the [^{51}Cr]EDTA end-point) were steep, and tended to shift towards lower doses with longer follow-up times. The dose-modifying factors defined as the dose needed to cause renal damage in 50% of the animals (ED_{50}) using single fraction TBI divided by the ED_{50} using fractionated TBI were 1.3 and 1.9 for the Fx 3 and Fx 9, respectively. These results may indicate that patients treated with TBI and BMT should be assessed for late kidney damage and that fractionation and particularly hyperfractionation may protect the kidneys from TBI-induced renal damage.

Key words: total body irradiation, fractionation, kidney, bone marrow transplantation
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INTRODUCTION

BONE MARROW transplantation is a life-saving treatment that is indicated in an increasing number of otherwise fatal malignancies and genetic disorders. However, it is a very toxic procedure with 10% treatment-related mortality even in experienced hands [1]. Recently, a relatively high incidence of late renal dysfunction after total body irradiation (TBI) and bone marrow transplantation (BMT) has been reported in a few retrospective studies [2–6]. Few experimental studies have focused on renal damage in animals treated with different TBI conditioning regimens and BMT [7–10]. Both the clinical and experimental data suggest that irradiation may be the major factor causing this renal damage. However, radiation is not the only nephrotoxic agent in this very complex treatment modality. Drugs such as cyclosporin A [11], viral [12] and fungal infections [13], and enhancement of radiation damage by cytotoxic agents [8, 14] may be contributing factors. However, the relative importance of each of these factors and the contributions made by other clinical variables, such as age and graft versus host disease, have not been systematically analysed. Thus, the available data on kidney damage after the complex procedure of BMT are far from being complete. Since most of the experimental studies on kidney damage after radiation have been performed by the use of isolated kidney irradiation and not TBI, we have initiated a study with the aim of investigating the effect of fractionated irradiation on late renal damage after TBI and BMT in a mouse model.

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MATERIALS AND METHODS

Animals

Male $C_3D_2F_1$/BOM mice were used and treated when 14–16 weeks of age. They were kept eight per cage under normal laboratory conditions and given tap water and food *ad libitum*. Light and darkness were adjusted to a 12-h cycle. The animals were ear marked and each animal was given a unique identification number.

Treatment

Unanaesthetised mice were restrained in acrylic jigs placed in a specially constructed acrylic box as described previously [15]. TBI was delivered with a 250-kV Phillips X-ray unit (10 mA,

2.2 mm copper half value layer (Cu HVL)), and the dose rate was 0.71 Gy/min.

Syngeneic bone marrow cells (2×10^6) were injected into the tail vein 4–6 h after the last treatment. The marrow was obtained from both femurs of normal untreated mice. The marrow cell suspension was diluted appropriately in Hank's balanced salt solution.

Assessment of kidney damage

[^{51}Cr]*EDTA clearance*. The single sample method described by Stewart and associates [16] was used with minor modifications. Ten microCuries of the radioactive material (in 0.1 ml) are injected per mouse intraperitoneally. Twenty minutes after injection, 75 μl of blood were withdrawn from the retro-orbital sinus in a capillary tube. The residual activity of the material was calculated as percentage of injected material in 20 μl of plasma.

The haematocrit (Hct). The %Hct values were measured after centrifugation of the blood samples taken during the chromium test. The values were determined using a Hawksley Micro-Haematocrit Reader.

Blood urea nitrogen (BUN). Blood samples for the urea nitrogen were taken from the retro-orbital sinus in a 125-μl non-heparinised capillary tube. The blood was centrifuged for 5 min at 64000 cycle/min, and 10 μl of plasma was examined in a Kodak Ektachrome DT60 analyser.

Experimental design and data analysis

TBI was given as three fractions in 3 days (fractionated, Fx 3) or as nine fractions in 3 days with interfraction interval of 8 h (hyperfractionated, Fx 9). In each of these TBI schedules, the animals were distributed between different dose levels. Control groups receiving BMT only were included. There were 24 animals in the control group and from eight to 16 animals in the other groups.

For each functional test a mean value of each group was calculated at each point of follow-up and plotted against time. To convert the values into quantal (all or nothing) data, each experimental group was then divided into responders and non-responders. Responders were defined as those animals in which the values for a specific functional parameter exceeded a predetermined (threshold) level. In all functional tests the threshold level was chosen to be values above three times the standard deviation of the mean value of an age-matched control group. All the animals that reached this threshold level (the responders) showed persistent and progressive damage until death or the end of follow-up period.

Based on the quantal response data, both time–response and dose–response data were generated. The time–response curves were calculated by the use of the Kaplan–Meier estimate for responders [17], while the dose–response data were computed by the use of a logit analysis [18]. The dose–response data were computed from the [^{51}Cr]EDTA data only and were used to calculate the iso-effective doses for the different schedules. The dose modifying factor (DMF) was defined as the ratio between the dose needed to cause response in 50% of the animals (ED_{50}) using a certain TBI protocol and the dose needed to cause the same effect when TBI was given as a single fraction. In the figures, the error bars represent one standard error.

RESULTS

Using [^{51}Cr]EDTA, kidney damage was evident after a dose of 14 Gy in the three fractions schedule (Fx 3) and 19 Gy in the nine fractions schedule (Fx 9) (Figure 1). Kidney damage progressed steadily with no recovery or a tendency to plateau. Both the level of kidney damage and the rate of progression were dose-dependent. The lower the dose per fraction, the higher the total dose at which the damage was observed.

Figure 2 shows the reduction of the %Hct against time after treatment. The %Hct detected damage at a lower dose than [^{51}Cr]EDTA and serum BUN. Figure 3 shows the elevation of the serum BUN against time after treatment for different dose levels. BUN detected kidney damage at the same dose levels as the [^{51}Cr]EDTA, i.e. 14 Gy and 19 Gy in Fx 3 and Fx 9,

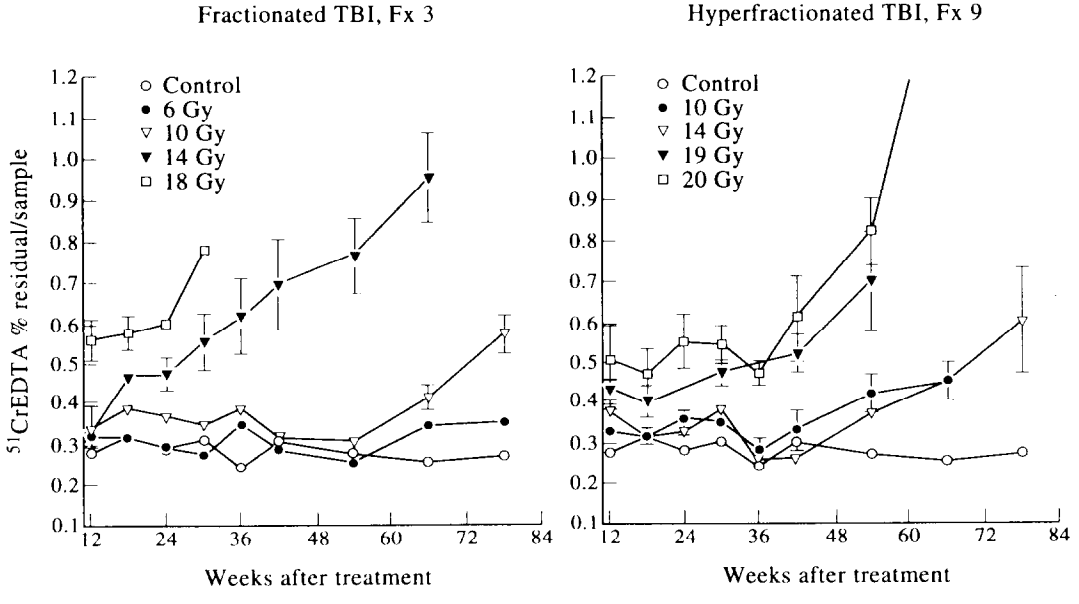

Figure 1. The change in the average residual activity of [^{51}Cr]EDTA after three or nine fractions total body irradiation (TBI). Error bars represent 1 S.E.

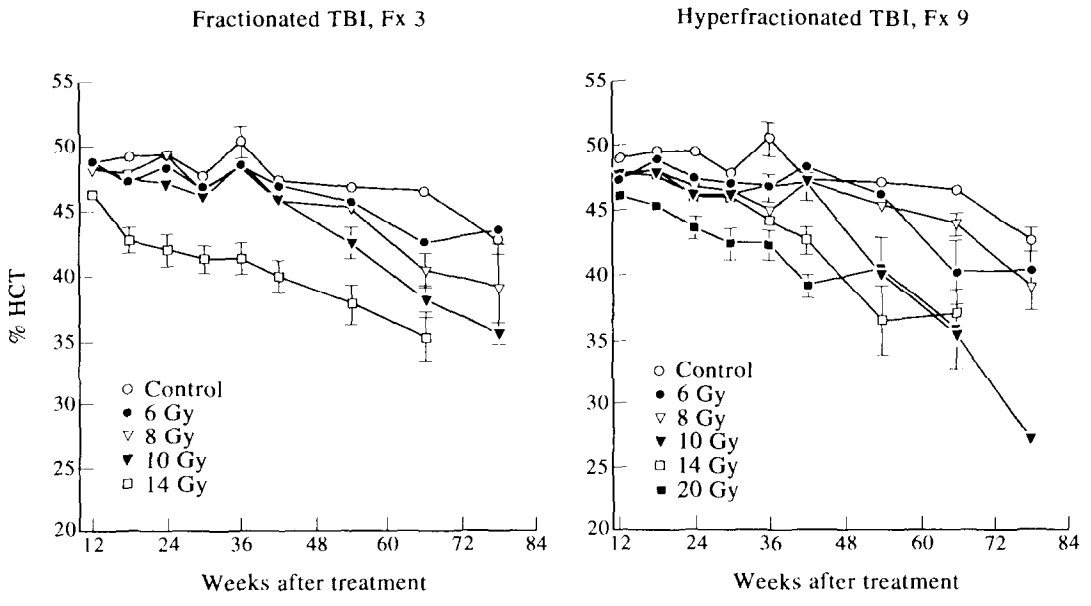

Figure 2. The change in the average percentage haematocrit (%Hct) after three or nine fractions total body irradiation (TBI). Error bars represent 1 S.E.

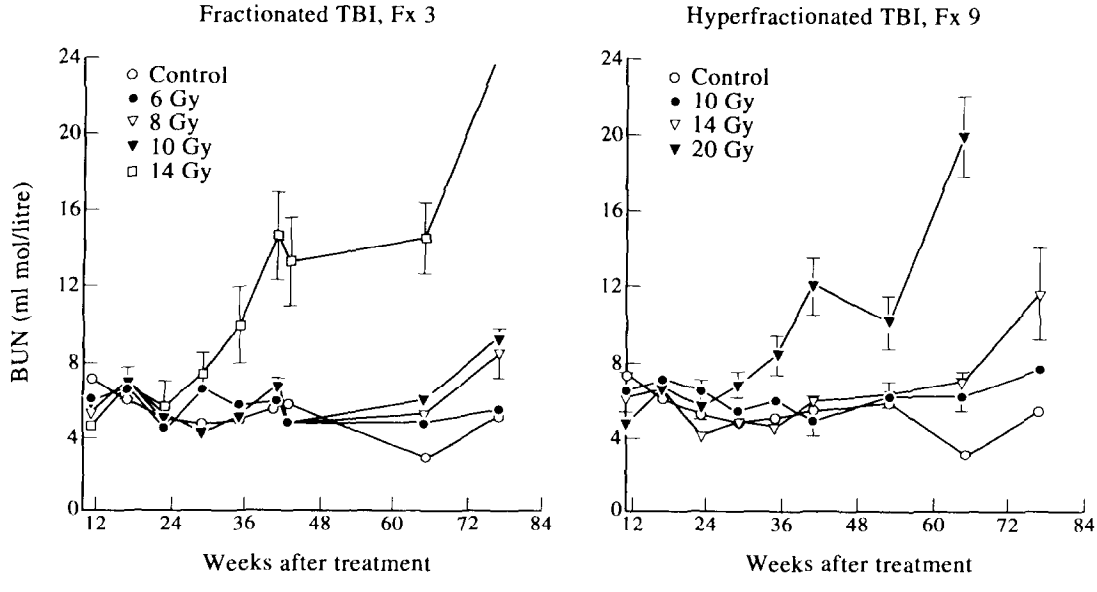

Figure 3. The change in the average blood urea nitrogen (BUN) after three or nine fractions total body irradiation (TBI). Error bars represent 1 S.E.

respectively. However, a longer latency was required. Kidney damage was evident around week 42 after treatment with both fractionation schedules.

Using the [^{51}Cr]EDTA assay, Figure 4 shows that, for any point in time there was an increase in the percentage of responding animals with an increase in the total dose for both Fx 3 and Fx 9 fractionation schedules. This reflects the reduction in the latency needed to cause a certain percentage of response as the total dose increased. Similar results are shown in Figure 5 using the percentage reduction in Hct, and with this end-point, responders were observed at doses as low as 6 and 8 Gy in both Fx 3 and Fx 9 schedules, respectively. However, at these low doses, the latency period needed before the expression of renal damage was more than a year after irradiation. To a lesser extent, BUN was also able to detect both an increase in the percentage of responding animals and a reduction in the latency before the expression of renal damage with increases in the total dose (Figure 6). However, BUN required a longer latency period before renal damage was expressed.

The dose–response curves generated for the two schedules demonstrated the shift to the left as the time after treatment increased from 18 to 24 weeks (Figure 7). They also showed the sparing effect of reducing the dose per fraction. The curves were shifted towards higher doses when the number of fractions increased from one to nine fractions (Figure 8). From the dose–response data, the ED_{50} and the DMF values for both the

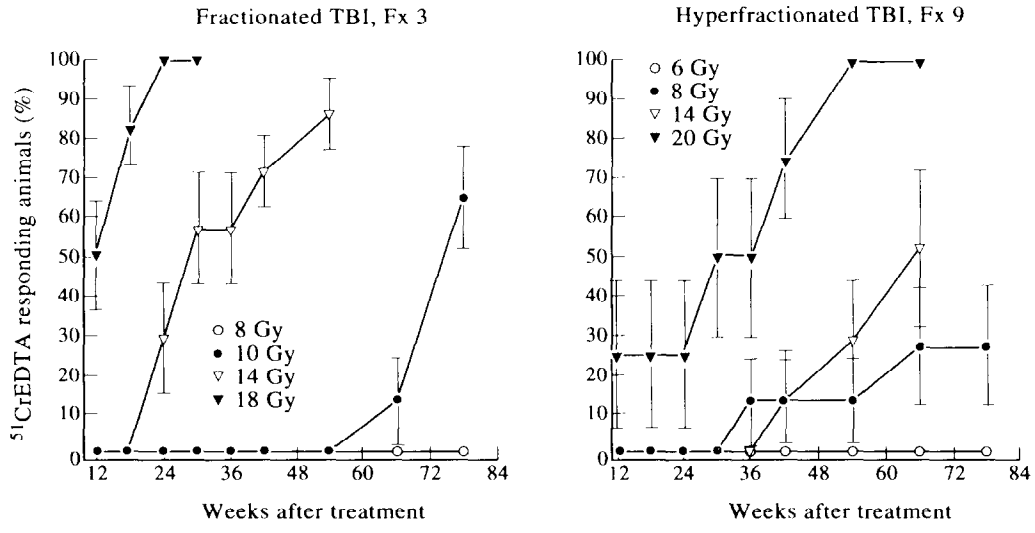

Figure 4. [^{51}Cr]EDTA time–response curves after three or nine fractions TBI. The percentages of responding animals were calculated using the Kaplan–Meier estimate. Error bars represent 1 S.E.

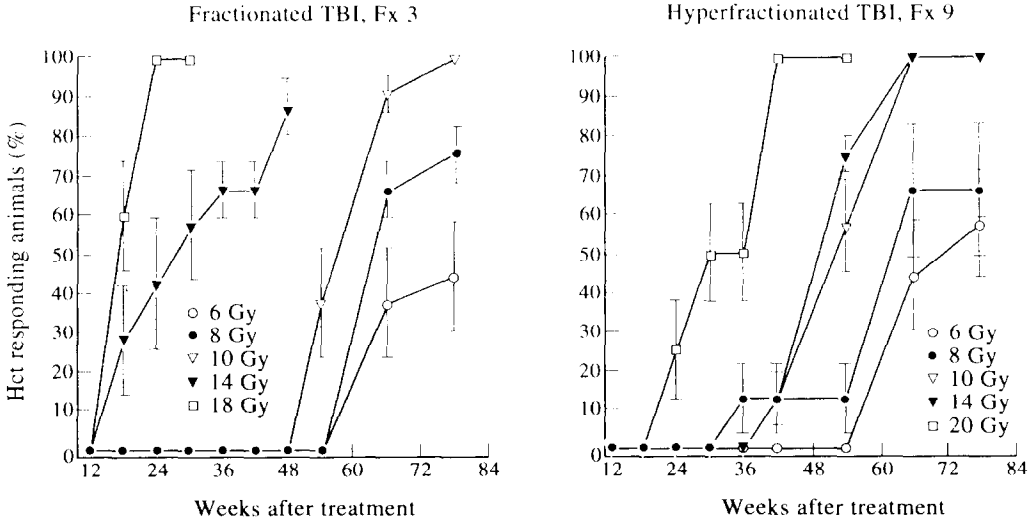

Figure 5. Haematocrit time–response curves after three or nine fractions total body irradiation (TBI). The percentage of responding animals were calculated using the Kaplan–Meier estimate. Error bars represent 1 S.E.

Fx 3 and Fx 9 schedules are shown at different weeks after treatment in Table 1. As seen, the Fx 9 schedule increased the ED_{50} more than the Fx 3 schedule. The DMF values remained constant from 18 to 30 weeks after treatment. The slope of the dose–response curves also remained constant from 18 to 30 weeks after treatment as the 10–90% response range was about 5 Gy in both fractionation schedules.

DISCUSSION

Despite its limitations, presenting the results as average values of the functional tests against time has the important value of demonstrating the progressive and increasing degree of damage, a criterion which the quantal analysis of data fails to demonstrate.

Using the residual activity of [^{51}Cr]EDTA in plasma, the minimal doses needed to cause renal dysfunction using the conventionally fractionated schedule (Fx 3) and the hyperfractionated schedule (Fx 9) were 14 and 19 Gy, respectively. Thus, hyperfractionation (with 8 h apart) could spare the kidneys more than conventional fractionation. This is in accordance with the data derived from localised kidney irradiation [19]. Since our schedules were not used in other studies it would be difficult to compare our results directly with others.

Neither age nor differences in body weight has influenced the results of the [^{51}Cr]EDTA or the BUN functional tests. However, because the %Hct showed consistent reduction with time in the control group (probably a normal physiological phenomenon in this animal strain), an age-matched control group was used for determining the threshold level of "responders" in all functional tests.

In agreement with the literature, the rate of progression of kidney damage was clearly dose-dependent [20]. Furthermore, the dependence of the latent period on the end point (%Hct etc.) and its shortening with higher radiation dose has been reported before with localised kidney irradiation [21].

The dose–response curves for kidney damage were steep. However, one should not compare different TBI schedules on

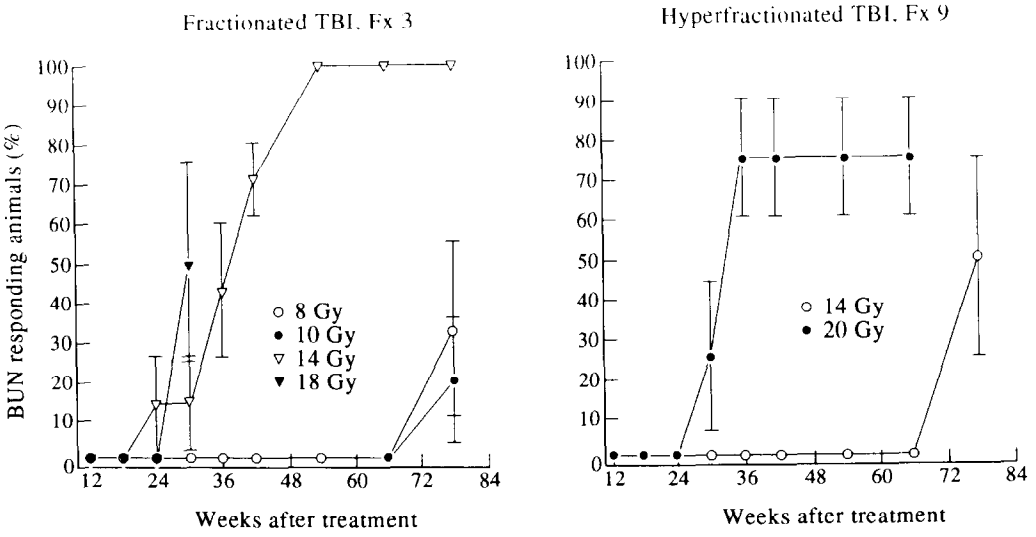

Figure 6. Blood urea nitrogen (BUN) time–response curves after three or nine fractions of total body irradiation (TBI). The percentages of responding animals were calculated using the Kaplan–Meier estimate. Error bars represent 1 S.E.

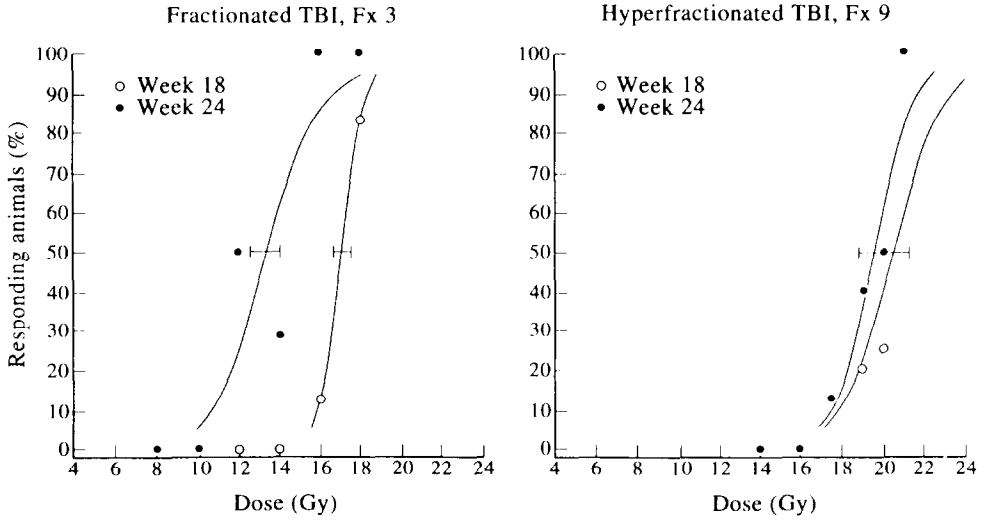

Figure 7. [^{51}Cr] EDTA dose–response curves at different weeks after three or nine fractions of total body irradiation (TBI). The graphs were generated using a logit programme. Error bars represent 1 S.E.

the basis of the slope of dose–response curves because of the few data points on the curves. Evaluation of published data suggests that the ED_{50} values of the [^{51}Cr]EDTA test in our study are lower than would be expected from localised bilateral kidney irradiation in mice. To some extent, this could be explained on the basis of the results of Moulder and colleagues [22] who showed that a single fraction of 9 Gy caused a higher degree of kidney damage when given as TBI than when given as localised kidney irradiation. However, comparing our results with other ED_{50} values published in literature should only be performed with care because of differences in the end-points, time of evaluation, animal strain and radiation schedule.

The sparing effect achieved by using fractionation has also been described using localised kidney irradiation. In our study, the hyperfractionation schedules were designed with an 8-h interfractional interval to guarantee the maximum repair of sublethal damage and the data showed that this interval provided a greater sparing effect than conventional daily fractionation. The fractionated schedule used in this study provided almost the same sparing effect as using TBI at a low dose rate (0.08 Gy/min) [23]. The DMF were 1.3 and 1.2 for the fractionated and low dose rate (LDR) TBI, respectively. The hyperfractionated schedule, on the other hand, provided a DMF of about 2. Thus, using hyperfractionated TBI is better for kidney protection than using LDR. Decreasing the dose rate below 0.08 Gy/min may provide even more sparing of kidney damage. However, the long treatment time needed at these very low dose rates will make this strategy impractical clinically.

The sensitivity of the Hct test in detecting renal damage has been described using localised kidney irradiation [19, 24]. It was suggested that the radiation sensitivity of the cells responsible for the secretion of erythropoietin in the kidneys is high. However, in our TBI model, other factors such as bone marrow suppression, radiation damage to the liver and gastrointestinal tract and chronic infections should also be considered as contributing to the development of anaemia.

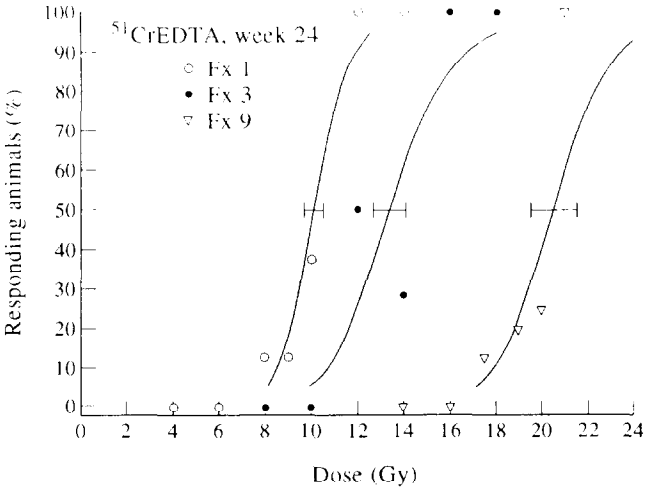

Figure 8. [^{51}Cr]EDTA dose–response curves at week 24 after a single fraction (Fx 1), three fractions (Fx 3) and nine fractions (Fx 9) of total body irradiation (TBI). The graphs were generated using a logit programme. Error bars represent 1 S.E.

Table 1. Dose–response data for renal damage evaluated by the [^{51}Cr] EDTA residual activity in mice at various weeks after total body irradiation (TBI) and bone marrow transplantation. TBI was given as a single fraction (Fx 1), fractionated (Fx 3) or hyperfractionated (Fx 9) treatment

	Fx 1	Fx 3		Fx 9	
Week	ED_{50} (Gy)	ED_{50} (Gy)	DMF	ED_{50} (Gy)	DMF
18	11.0 (0.4)	17.1 (0.4)	1.5 (0.1)	20.5 (1.1)	1.9 (0.1)
24	10.1 (0.4)	13.4 (0.7)	1.3 (0.1)	20.5 (1.1)	2.0 (0.1)
30	10.1 (0.4)	12.8 (0.6)	1.3 (0.1)	19.5 (0.6)	1.9 (0.1)

Values in parentheses are ±1 S.E. DMF, dose-modifying factor.

Interstitial pneumonitis was carefully monitored in these animals. Although both kidney and lung damage coexisted in some animals, there was a dose range at which only kidney damage occurred. A similar observation was demonstrated with single fraction TBI given at either high or low dose rate [23].

Clinically, renal damage after BMT is now becoming an increasingly important late complication. In the present study, we have demonstrated that, in mice, radiation-induced kidney damage after TBI and BMT is a potentially lethal dose-limiting complication. It seems to attain the same biological criteria as after localised kidney irradiation but at lower radiation doses.

Although it is difficult to extrapolate these results to the clinical situation, the data indicate that it would be prudent to carefully assess the patients treated with TBI and BMT for late kidney damage. We recommend the use of a hyperfractionated TBI schedule with long interfraction intervals for maximum kidney sparing and the use of a sensitive test using radionuclides to monitor late renal damage.

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