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SKIN REACTIONS IN MOUSE BY FRACTIONATED NEUTRON IRRADIATIONS WITH THE SAME NSD_n

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Since the failure of fast neutron therapy about forty years ago (STONE et coll. 1940, STONE & LARKIN 1942), it was considered disadvantageous by virtue of the excessive late effect on normal tissues (STONE 1948), until it was revealed that excess doses were given to STONE's patients (SHELINE et coll. 1971, BEWLEY et coll. 1963) and that fast neutrons, having high linear energy transfer (LET), could be effective for killing hypoxic cells in tumours because of their low oxygen enhancement ratio (OER) (GRAY et coll. 1953, THOMLINSON 1963, ALPER 1963, FOWLER et coll. 1963). On this basis, radiation therapy with cyclotron-produced neutrons was begun again at the Hammersmith Hospital in England and also in the United States and Japan during the past decade.

The neutron therapy was planned also in this institute in 1970, and its irradiation regimen was two fractions a week, due to a limited availability of machine time. It was, therefore, required that the effect of neutron irradiation on normal tissues, especially the skin, had to be determined for this new dose fractionation, because little was known of its effect and because normal tissue tolerance determines the irradiation dose.

The experiments were performed to analyze three different dose fractionations (two fractions a week), from which NSD_n of normal tissues was calculated by means of the Hammersmith Hospital method. According to Ellis' formula for neutrons (FIELD 1972), the effect of these fractionations would not differ, but experiments seemed necessary for a confirmation of the validity of these new fractionations

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Table

Dose fractionations given two times a week with the same NSD_n

Group	Total dose (Gy)	Dose per fraction (Gy)	No. of fractions	Time (days)
I	15.00	1.25	12	39
II	14.60	1.46	10	32
III	14.08	1.76	8	25

before they were used as therapy regimens. The therapy was started in November, 1976 (IINO & KUMASAWA 1977). Apart from the therapeutic use, the effects of single dose of fast neutrons, roentgen rays and ^{60}Co γ-rays were also examined.

Materials and Methods

To obtain the new fractionations given two times a week and equivalent to the Hammersmith Hospital method, i.e. 14.4 Gy (1 440 rad according to the 'old Hammersmith rad', which was devalued by a factor of 1.08, SMITH et coll. 1975) given in 12 treatments over 26 days (CATTERALL 1974), the following ELLIS' formula for neutrons was used:

$$\text{Total dose} = NSD_n \times N^{0.04} \times T^{0.11}$$

where NSD_n is the nominal standard dose for fast neutrons, N is the number of fractions and T is the over-all time in days. The new fractionations calculated are given in the Table.

The assessment of radiation-induced injury to the skin was carried out with the skin transplantation method of KAL et coll. (1974) with slight modifications. The hair of skin of donor and recipient mice was removed with a hair-clipper and a depilatory a few days before transplantation. The skin field, about 17 mm in diameter, on the back of white DDD mice was irradiated with fast neutrons through two collimators for therapy and for this experiment and according to the fractionation regimen given in the Table. Circular pieces of the skin, 10 mm in diameter, were excised and placed on the back of the recipient mice, in which graft beds had been prepared by excision of similar but slightly smaller pieces of skin. The transplantation was performed by rubbing edges of the skin with very small amounts of α-cyanoacrylate. The grafted skin pieces were covered with sterilized gauze and then fixed with vinyl tape and pressure elastic grip bandage for one to two weeks. Brown (DDD × C3H/He)F_1, hybrid mice were used as recipients to avoid immunologic complication and to distinguish the grafted skin clearly. The ratio of the treated and untreated areas of grafted skin remaining for three months was used as a parameter

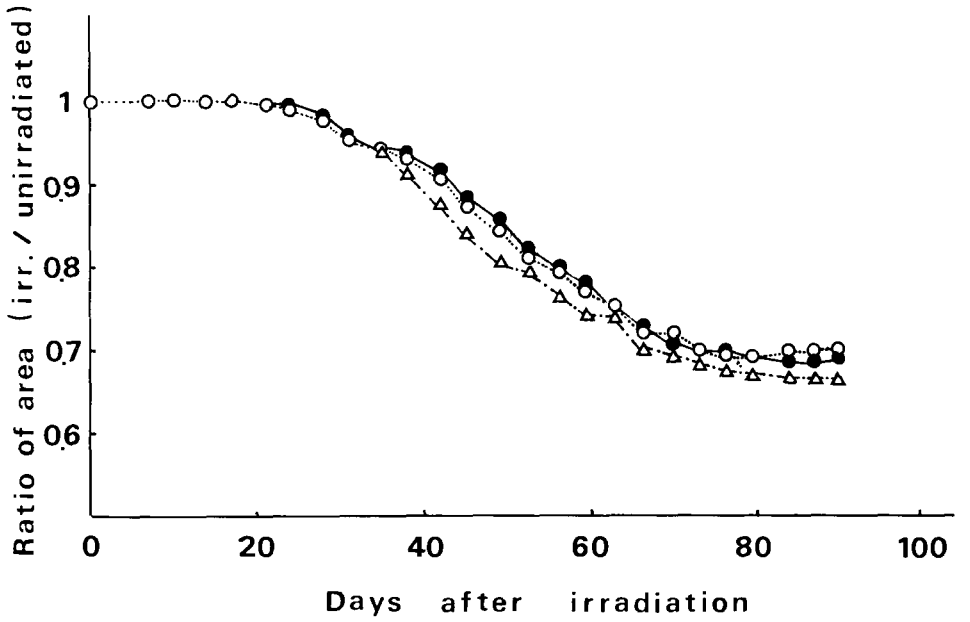

Fig. 1. Reductions of the area of skin grafts by fractionated neutron irradiation relative to that of unirradiated ones. A dose per fraction of each group is 1.25 Gy (○), 1.46 Gy (●) and 1.76 Gy (△). Cf. the table for details of the fractionations. For clarity, representation of standard deviations is omitted in the figure, but the differences among the 3 groups are insignificant.

for skin injury. Part of the skin-irradiated mice had been bred for a certain period for observation of possible change in the irradiated skin.

Fast neutrons ($\bar{E}_n = 6$ MeV) with a dose rate of 200 mGy/min were produced by bombarding the thick beryllium target with 50 μA of 15 MeV deuterons accelerated with the cyclotron of the institute. Roentgen rays were generated by a therapy machine operated at 200 kV and 25 mA and were filtered through 0.3 mm Cu and 1 mm Al. The dose rate was 900 mGy/min. ^{60}Co γ-rays were obtained from a therapy unit at a dose rate of 384 mGy/min. Irradiation of the skin by these types of radiation was performed without the use of build up materials.

Results

The ratio of the irradiated skin graft area to that of unirradiated controls appears in Fig. 1. A decrease began about three weeks after the irradiation and approached constant value after about 70 to 80 days. No significant differences were observed between the three fractionations (1.25, 1.46 and 1.76 Gy per fraction). The result suggests that a fractionation two times a week, even with a high dose per fraction is therapeutically applicable at least from the standpoint of the skin reaction of mice, since the observed slight injury may be considered permissive. However, it remains

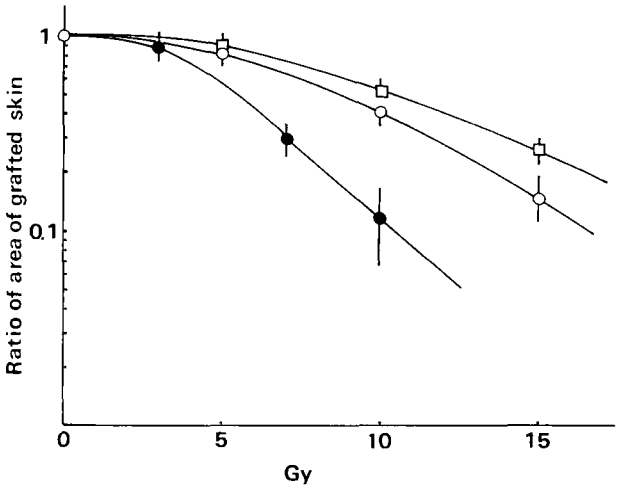

Fig. 2. Reductions of the area of skin grafts irradiated with single doses of fast neutrons (●), roentgen rays (○) or ^{60}Co γ-rays (□) relative to that of unirradiated ones.

uncertain whether the mouse skin (especially the basal cells of epidermis), responds as does the human skin to fast neutrons, although similar RBE has been reported (FIELD & HORNSEY 1971). The result also demonstrates the applicability of Ellis' formula for fast neutrons.

High single doses of fast neutrons, roentgen rays and ^{60}Co γ-rays, produced marked differences in skin injury (Fig. 2). The injury was slight at a dose of 3 Gy of fast neutrons, but increased gradually at higher doses. Roentgen rays and ^{60}Co γ-rays also gave qualitatively similar, but quantitatively lesser effects on the skin. ^{60}Co γ-rays were least effective. The doses at which 50 per cent of the skin was injured were 5.5, 8.5 and 10.5 Gy of fast neutrons, roentgen rays and ^{60}Co γ-rays, respectively. These lead to the RBE values of fast neutrons and ^{60}Co γ-rays for 50 per cent of skin injury being 1.6 and 0.8, respectively. The values mean that the skin impairment with fast neutrons is less than that with roentgen rays, as has been expected from their build-up, because the RBE of fast neutrons for most normal and malignant cells are 2 to 3 at 50 per cent level of surviving fractions.

Discussion

As predicted from Ellis' formula, the result showed that for a given total dose the injury to the skin irradiated two times a week was small. Moreover, the effect of a high dose per fraction was not different from that of a low dose per fraction. This is in good accordance with the death of mice from radiation pneumonitis (HORNSEY et coll. 1975), and implies the possibility of a therapy with a high dose per fraction for a short period. Likewise, a high dose per fraction is known to be superior, because small numbers of hypoxic cells in tumours are not sterilized effectively at lower doses (FOWLER & MORGAN 1963). However, if a high dose per fraction is adopted in the therapy, it is strongly required that the dose per fraction be increased step by step with attention to skin impairment.

Two points must be discussed further. First, the present work was performed according to the 'old Hammersmith rad'. Thus 15.6 Gy (1 560 rad) of total dose, instead of 14.4 Gy, should have been used to calculate NSD_n. For the present purpose it seemed inadequate that, consequently, NSD_n of normal tissues was underestimated in the experiment. However, as the skin injury occurred even under the condition of less NSD_n (Fig. 1), the result would be significant not only in terms of the intercomparison of the three different types of radiation which had the same 'NSD_n' (ORTON & ELLIS 1973), i.e., 92 per cent of NSD_n, but also in comparison with other treatments, e.g., three fractions a week. Secondly, the neutron dose used includes γ dose, as does United States neutron dose, while the Hammersmith neutron dose does not. However, the difference can almost be ignored in terms of biologic effectiveness for the following reasons: (1) contamination of neutrons with γ-rays is only a few per cent at the tissue surface, and (2) RBE of γ-rays is about three times lower than that of neutrons at low doses.

Most work on injury to the skin has been carried out using the method of scoring the skin reaction by direct observation, but little work has been done using skin transplantation (KAL et coll.). For this reason the method of skin transplantation was adopted in the present work, although the relation between the two methods is not well understood. However, it may be likely that either the extent of injury or the RBE produced by the methods differ, because, for example, the RBE for skin injury appeared lower as evaluated by the method of transplantation than by scoring.

A high single dose affected the skin more than did the fractionated doses. This could possibly be due to occurrence of repair and repopulation in the intervals of and after fractionated irradiation (DENEKAMP & FIELD 1974).

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SUMMARY

The results of three fractionations of neutron irradiation with the same NSD_n were examined for skin reactions in mice. No significant differences were observed between these fractionations given two times a week (15 Gy/12 fractionations/39 days, 14.6 Gy/10 fractionations/32 days and 14.08 Gy/8 fractionations/25 days). The result suggests the value of a high dose per fraction given two times a week.

ZUSAMMENFASSUNG

Die Resultate der Fraktionierung von drei Neutronenbestrahlungen mit der gleichen NSD_n wurden hinsichtlich der Hautreaktionen von Mäusen untersucht. Keine signifikanten Unterschiede zwischen diesen Fraktionierungen, die zweimal wöchentlich gegeben wurden (15 Gy/12 Fraktionen/39 Tage, 14,6 Gy/10 Fraktionen/32 Tage, 14,08 Gy/8 Fraktionen/25 Tage), wurden beobachtet. Die Ergebnisse deuten auf den Wert einer hohen Dosis pro Fraktion, zweimal wöchentlich verabfolgt, hin.

RÉSUMÉ

Trois fractionnements de dose de neutrons avec la même NSD_n ont été examinés en ce qui concerne les réactions cutanées de la souris. L'auteur n'a pas trouvé de différence significative entre ces fractionnements donnés deux fois par semaine (15 Gy/12 fractions/39 jours, 14,6 Gy/10 fractions/32 jours et 14,08 Gy/8 fractions/25 jours). Ce résultat fait penser qu'il serait intéressant de donner une forte dose par fraction administrée deux fois par semaine.

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