Growth, endocrine function and quality of life after haematopoietic stem cell transplantation

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EFFECTS OF TOTAL-BODY IRRADIATION ON GROWTH, THYROID AND PITUITARY GLAND IN RHESUS MONKEYS

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Abstract

Aim: To investigate the effect of total-body irradiation (TBI) on growth, thyroid and pituitary gland in primates.

Materials and methods: Thirty-seven rhesus monkeys (mean age 3.1 ± 0.6 years) received either a low-dose (4 - 6 Gy) TBI (n = 26) or high-dose (7 - 12 Gy) TBI (n = 11) and were sacrificed together with 8 age-matched controls after a post-irradiation interval of 5.9 ± 1.5 years. Anthropometric data were collected; thyroid and pituitary glands were examined; serum levels of thyroid stimulating hormone (TSH), free thyroxin (FT4), insulin-like growth factor-I (IGF-1) and its binding protein-3 (IGFBP-3) were measured.

Results: Decrease in final height due to irradiation could not be demonstrated. There was a dose-dependent decrease in body weight, ponderal index, skinfold thickness and thyroid weight. The latter was not accompanied by elevation of TSH or decrease in FT4. Structural changes in the thyroid gland were found in 50% of the irradiated animals. Levels of IGF-I and IGFBP-3 did not differ between the dose groups, but the high-dose group had a lower IGF-I/IGFBP-3 ratio.

Conclusions: Total body irradiation had a negative effect on body fat. There was no evidence of (compensated) hypothyroidism, but dose-dependent decrease in thyroid weight and changes in follicular structure suggest some effect of TBI on the thyroid gland. The decreased IGFI/IGFBP-3 ratio in the high-dose group can indicate that the somatotrophic axis was mildly affected by TBI. These results show that TBI can have an effect on the physical build and thyroid gland of primates even in the absence of cytostatic agents or immunosuppressive drugs.

Introduction

Total-body irradiation (TBI) is frequently used in combination with high-dose chemotherapy in conditioning regimens for haematopoietic stem cell transplantation (HCT). Unfortunately this aggressive conditioning has negative effects on several organs including those of the endocrine system [1]. Examples of negative effects on endocrine organs are decreased fertility and hypogonadism, (compensated) hypothyroidism, and growth hormone deficiency (GHD), respectively [2]. In children, TBI and HCT often result in impaired growth and reduction of final height [3;4]. Although TBI is considered to be an important etiologic factor in these disorders, its exact role is difficult to assess due to the superimposed and/or synergistic effects of other factors used in clinical settings (e.g. cytostatics, antimicrobial drugs, GVHD, corticosteroids and the underlying disease itself). Therefore, studies in animals without (previous treatment of) an underlying disease are helpful in the investigation of TBI as a single toxic factor. Radiation experiments in primates are of relevance since the response to radiation in monkeys does not seem to be significantly different from that in man. This has been demonstrated for acute effects on the haematopoietic system [5] and late effects such as tumour induction [6]. Furthermore an outbred species such as the rhesus monkey (Macaca mulatta) is more representative as an animal model to assess the effect of TBI as a single toxic factor than are inbred rodents. Results of the effect of TBI, without interference of other medication such as cytostatics or immuno-suppressive drugs, on the eye [7] and on hepatic and renal function [8] are already available. This article describes the effect of TBI as a single toxic factor on both the thyroid gland and somatotrophic axis.

Materials and methods

Animal population

Between 1963 and 1989 approximately 100 rhesus monkeys received TBI in the Biomedical Primate Research Centre (BPRC) of Rijswijk, the Netherlands, in experiments on both the efficacy of the HCT procedure and the late effects of TBI. In a terminal experiment 37 irradiated animals and 20 control animals

were sacrificed. Anthropometric measurements were taken and after anaesthesia (using ketamin and vetrancyl) the animals were heparinised and euthanised. Tissue samples gathered during autopsy were distributed to several research institutes interested in late effects of TBI. We were able to obtain blood samples and tissue samples of the thyroid and pituitary gland. The mean age of the irradiated animals was 8.9 ± 1.6 years (range 6.2 - 11.8). There were eight age-matched control animals with a mean age of 8.9 ± 2.0 years (range 6.8 - 12.5). The mean age of the control animals that were excluded from the analyses was 24.9 ± 5.8 years (range 17.8 - 34.8). Total body irradiation was given at a mean age of 3.1 ± 0.6 years (range 2.0 - 4.6), which means that most of the animals had just entered puberty at the time of the TBI. Age at time of irradiation did not differ between the low-dose group (LD) and the high-dose group (HD). The post-irradiation interval (overall 5.9 ± 1.5 years) was longer in the high-dose group compared to the low-dose group: median 6.8 (4.8 - 8.3) years versus 5.7 (3.3 - 7.4) years (P = 0.003). All animals involved in the present study were bred within the BPRC colony and kept under identical housing conditions. They were fed commercial food pellets (Hopefarms) and a diet of fresh fruit and vegetables. The animals were procured, maintained and used in accordance with Dutch law and regulations, the Animal Care and Use Committee and the Animal Ethical Committee approved all experiments.

Irradiation and additional treatments

The total-body irradiations were performed at an instantaneous dose-rate of 0.3 Gy min^{-1} with either 300 kV or 6 MV X-rays. Monkeys were irradiated in a specially designed cage that was slowly rotated along its longitudinal axis in the beam in order to obtain an optimal dose distribution over the animal. At a later stage the animals were irradiated bilaterally. Radiation doses received by the monkeys are expressed as absorbed dose in soft tissue averaged over the animal [9]. Three groups of animals were distinguished: control animals (n = 8), animals that received a relatively low TBI dose (4 - 6 Gy as a single fraction; n = 26), and those which received a high TBI dose (7 - 8.5 Gy as a single fraction; n = 8, or 12 Gy in two single fractions on two consecutive days; n = 3). The animal characteristics in the different dose groups are presented in table 1. Both the TBI doses and dose rate correspond well with those used in our clinic in several hundred patients transplanted at our centre since 1965. Patients

transplanted for severe aplastic anaemia, immune-deficiency syndromes or haemoglobinopathies received 4 - 5 Gy single fraction TBI. Patients with haematological malignancies receive either 7 - 8 Gy as a single fraction, or 12 Gy in two single fractions on two consecutive days (instantaneous dose rates used are always 0.25 ± 0.05 Gy min^{-1}).

Table 1. Number of male and female monkeys in subsequent dose categories

TBI dose	Males	Females
Control animals (n=8)	2	6
Low-dose TBI (n=26)		
4.0 Gy	3	-
5.0 - 5.3 Gy	15	5
6.0 Gy	3	-
High-dose TBI (n=11)		
7.0 Gy	1	-
8.0 Gy	2	1
8.5 Gy	3	1
2 x 6.0 Gy	2	1

After TBI seven animals (all LD) received supportive care only, the other animals received additional treatment to enhance the recovery of bone marrow. Additional treatment consisted of either cytokines only (n = 18, one HD), or HCT (n = 5, all HD), or both HCT and cytokines (n = 7, five HD). Cytokines used were human granulocyte macrophage colony-stimulating factor (GM-CSF), rhesus monkey interleukin-3 (IL-3) or rhesus monkey interleukin-6 (IL-6) for approximately 14 days.

Morphological, functional and histological assessments

Anthropometrical measurements consisted of body weight, subscapular skinfold thickness, upper leg length and lower leg length, sitting height and

head circumference. All measurements were done according to standard protocols[10]. Total length was estimated by adding sitting height, upper leg length and lower leg length. Ponderal index was calculated as (body weight x 100) / sitting height[11]. Thyroid status was evaluated by determining thyroid weight, serum levels of free thyroxin (FT4) and thyroid stimulating hormone (TSH). Both FT4 and TSH were assayed at the Leiden University Medical Centre; FT4 was measured by radio-immunoassay (RIA) and TSH by immuno-radiometric assay (IRMA), (both from DPC, Los Angeles CA). The thyroid glands of 26 irradiated (seven high-dose TBI) and eight control animals were sectioned and stained at the Veterinary Faculty of Utrecht University with haematoxylin-eosin (HE), periodic-acid-Schiff (PAS), and immunohistochemical staining using antibodies against calcitonin. The somatotrophic axis was evaluated by measuring serum levels of insulin-like growth factor-1 (IGF-I) and its binding protein 3 (IGFBP-3) (assayed at the Wilhelmina Children's Hospital, Utrecht[12]). Sections of the pituitary glands were stained with HE, PAS, orange-G and immunohistochemical staining using antibodies against growth hormone.

Statistical analyses

Results are expressed as mean (SD) or median (range) as indicated. The results of the different dose groups were compared by non-parametric tests (Mann-Whitney U test and Kruskal-Wallis H test). Dose dependency was analysed by Spearman's correlation for both sexes separately. Linear regression analysis with calculation of partial correlation coefficients was done on the total group of all animals controlling for possible confounding factors such as sex, age and body weight. All analyses were performed using two-sided tests.

Results

Anthropometric measurements

Because of the differences between both sexes, separate analyses were done for each sex. Table 2 shows the results of the anthropometric measurements.

Table 2. Auxological results in the different dose groups. Data presented as mean (SD).

	MALES				FEMALES			
	Reference	Control (n=2)	Low-dose (n=21)	High-dose (n=8)	Reference	Control (n=6)	Low-dose (n=5)	High-dose (n=3)
Body weight (kg)*	10.8	7.8 (0.5)	7.0 (1.2)	5.9 (0.8)	8.7	4.9 (0.8)	4.5 (0.7)	3.8 (0.5)
Ponderal index**	19.0	14.0 (1.6)	12.9 (1.7)	11.3 (1.3)	16.8	10.2 (1.0)	9.3 (1.1)	8.0 (0.7)
Skinfold (cm)*	4.1	4.0 (1.4)	3.5 (1.4)	2.6 (0.8)	5.4	4.3 (0.8)	2.5 (0.7)	2.3 (1.2)
Sitting height (cm)*	56.8	56.1 (2.8)	53.8 (3.0)	52.4 (2.3)	51.9	47.5 (3.5)	48.2 (1.4)	47.3 (2.6)
Upper leg length (cm)	n.a.	18.5 (2.3)	18.5 (1.3)	18.1 (0.9)	n.a.	15.0 (1.0)	15.5 (0.6)	15.9 (1.2)
Lower leg length (cm)	n.a.	21.8 (0.4)	21.0 (1.5)	20.6 (1.5)	n.a.	17.9 (0.7)	18.1 (0.8)	19.3 (1.3)
Head circumference (cm)	n.a.	29.5 (0.1)	30.1 (1.9)	28.9 (1.7)	n.a.	26.4 (2.0)	26.8 (0.8)	26.4 (1.3)
Estimated height (cm)	n.a.	96.3 (0.1)	93.3 (5.1)	91.1 (3.1)	n.a.	80.4 (4.0)	81.7 (2.2)	82.5 (4.7)

* Reference values are means, based on data from Schwartz and Kemnitz[13] on 6-14 year old animals.

** Calculated from mean values of body weight and sitting height derived from data of Schwartz and Kemnitz[13].

n.a. Not available.

Comparison of the results for the different dose groups revealed a decrease with increasing TBI doses in body weight in males P = 0.036), a decrease in ponderal index in both males (P = 0.045) and females (P = 0.033), and a decrease in subscapular skinfold thickness in females (P = 0.018). Radiation-dose dependency was confirmed by correlation analyses for body weight (males: r = -0.37; P = 0.042 and females: r = -0.57; P = 0.033), for ponderal index (males: r = -0.37; P = 0.039 and females: r = -0.69; P = 0.006), and for skinfold thickness in females (r = -0.65; P = 0.013). After controlling for the effects of sex and age, the partial correlation coefficient between body weight and TBI dose was r = -0.51 (P = 0.001), between ponderal index and TBI dose r = -0.55 (P < 0.001) and between skinfold thickness and TBI dose r = -0.39 (P = 0.011). The other parameters, including sitting height and estimated total length did not show significant differences between the dose groups, nor were there indications for radiation-dose dependency of those parameters.

Functional and morphological evaluation of the thyroid gland

The mean thyroid weight decreased with increasing TBI dose (table 3; P < 0.001). There was a negative correlation between thyroid weight and TBI dose (r = -0.59; P < 0.001), which remained after controlling for body weight (partial r = -0.51; P = 0.001). Free T4 and TSH levels did not differ between the various dose groups. Table 3 (upper part) summarises the results of the thyroid evaluation.

Table 3. Results of thyroid and somatotrophic evaluation. Organ weights as mean (SD); serum parameters as median (range).

	Control	Low-dose TBI	High-dose TBI
Weight thyroid (g)	0.59 (0.16)	0.47 (0.16)	0.30 (0.10)
TSH (mIU/L)	0.14 (0.01-2.41)	0.35 (0.37-1.72)	0.42 (0.12-2.44)
Free T4 (pmol/L)	4.2 (1.8-13.1)	6.9 (2.2-18.1)	5.8 (3.3-10.0)
Weight pituitary (g)	0.096 (0.024)	0.084 (0.018)	0.082 (0.017)
IGF-1 (mg/L)	0.37 (0.23-0.62)	0.40 (0.21-0.88)	0.32 (0.22-0.57)
IGFBP3 (mg/L)	1.55 (1.01-3.74)	1.88 (1.17-2.44)	1.71 (1.30-2.03)
IGF-1/IGFBP3 ratio	0.19 (0.16-0.34)	0.21 (0.12-0.47)	0.17 (0.12-0.23)

On histological examination 13 of the 26 irradiated monkeys (six out of seven in the high-dose group and seven out of 19 in the low-dose group) had small follicles (which were lined with higher cuboidal epithelium) compared to the non-irradiated animals. An example of these differences is shown in figure 1.
Animals with these structural changes had a lower thyroid weight compared to animals without these changes (mean: 0.522 versus 0.354 g; P = 0.004) and there was a correlation between TBI dose and the presence of structural changes (r = 0.54, P = 0.001). One irradiated monkey (TBI dose 5.0 Gy) had a macrofollicular goitre with focally distinct papillary hyperplasia. The papillae and some newly formed follicles had high columnar cells and many resorption vacuoles were present. The distended follicles had flat epithelial cells. No other histological abnormalities were found in the thyroid sections.

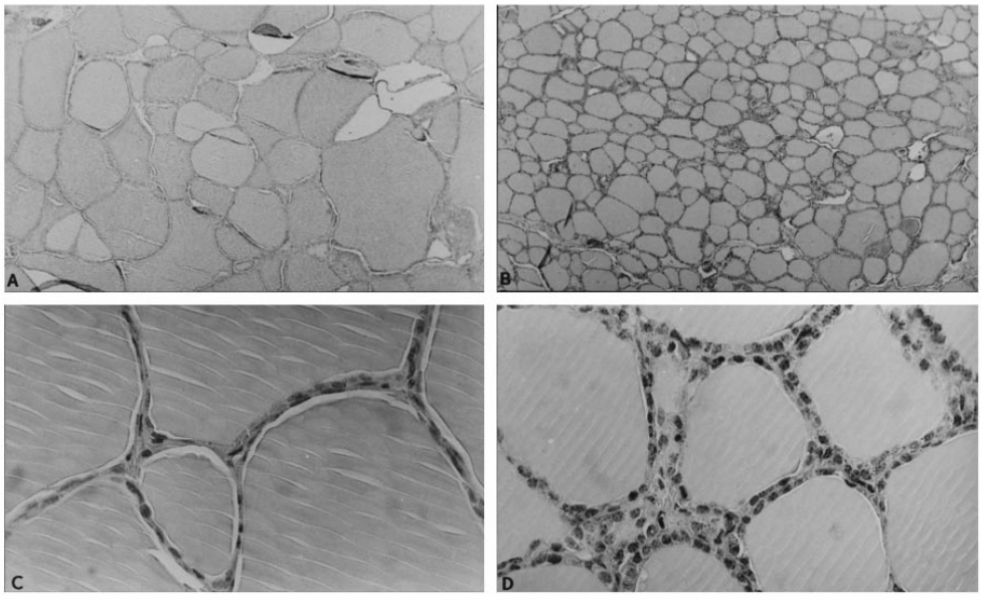

Figure 1. Example of the typical thyroid abnormalities found in irradiated monkeys (left side) compared to the normal thyroid tissue of a non-irradiated monkey (right side). Note the decrease in follicle size lined with higher cuboidal epithelium in the irradiated animal (staining: Haematoxylin-Eosin; magnification: a & b: 10x5; c & d: 10x40).

Functional and morphological evaluation of the somatotrophic axis

Results of evaluation of the somatotrophic axis and the pituitary gland are included in table 3 (lower part). Pituitary weight did not differ between the various dose groups. No correlation was found between TBI dose and pituitary weight even after controlling for body weight and sex. The plasma levels of IGF-I and IGFBP-3 did not differ between different dose groups. However, the ratio of IGF-I and IGFBP-3 was higher in the low-dose group compared to the high-dose group ($P = 0.047$). On histological examination no abnormalities were found in the pituitary glands apart from an area with hyperplasia of prolactin-producing cells in one irradiated monkey (TBI dose 8.5 Gy).

Discussion

Studies on the effect of TBI in HCT patients are complicated by confounding variables such as the initial disease, the use of cytostatics in both initial

treatment and conditioning regimens, the complications of allogeneic marrow transplantation and the effects caused by post-HCT medication (e.g. corticosteroids, cyclosporine A and antibiotics). The animals in this study therefore offer a unique possibility to investigate the effects of TBI as a single toxic agent in primates. Compared to the measurement data published by other authors[11;13] the animals used as controls in this study had a relatively low body weight and ponderal index, and the females had low sitting heights and subscapular skinfold thickness. In spite of this, though, body weight, ponderal index and subscapular skinfold thickness were clearly influenced by TBI: the high-dose irradiated animals were skinny compared to age-matched non-irradiated animals. A possible explanation for these changes are provided by Griffiths et al.[14]. They describe alterations in gastrointestinal regulatory peptides in the irradiated monkeys, which could have resulted in anorexia. In contrast to the observations in children after TBI and HCT, no effect of TBI on growth could be found in the present study. The loss of height potential in children is most prominent during puberty, as many patients have an impaired pubertal growth spurt[3;15]. Rhesus monkeys exhibit only a small pubertal growth spurt compared to humans[16], and therefore, impairment of this growth spurt would not have major effects on final height. Sonneveld and van Bekkum[17], however, showed that TBI (as single toxic agent) can cause inhibition of growth in rhesus monkeys from the same colony as the animals in the present study. Radiation doses of 7.5 Gy or higher were required and the effect was more pronounced in animals irradiated before the age of 40 months. The relatively low-doses used in most animals in our study and the relatively high ages at the time of irradiation could explain the lack of growth impairment (at the age of three the animals have attained approximately 90% of their adult sitting height)[10]. The difference in thyroid weight between the various dose-groups and the negative correlation between thyroid weight and TBI dose suggest an effect of TBI on the thyroid gland. Small follicles lined with higher epithelium, as found in thyroid glands of irradiated animals, are usually associated with a high activity of the follicular epithelial cells. We speculate that, in some animals, radiation has induced damage to thyrocytes which is compensated by their increased activity. This increased activity may have resulted in a decreased colloid content, which, in conjunction to a possible reduction in cell number, could explain the decrease in thyroid weight. In humans receiving a TBI and HCT for haematological malignancies, (compensated) hypothyroidism occurs

in 15 - 50% of the patients[18]. The incidence of radiation-induced (compensated) hypothyroidism depends on radiation dose, fractionation schedule and post-irradiation interval[19]. The small number of animals in the high-dose group and the limited post-irradiation interval could explain why we did not find hypothyroidism. The incidence of malignant tumours of the thyroid gland is increased after irradiation[20], and young patients with papillary thyroid carcinoma often have a history of irradiation of the thyroid gland[21;22]. After HCT and TBI, patients are at risk for secondary malignancies[23;24], and although most of those are of haematological origin[25;26] the incidence of thyroid carcinomas is also increased[23]. In this study no evidence for malignant thyroid tumours was found in any of the irradiated monkeys. This could, however, be caused by the relatively short post-irradiation interval, as radiation induced (thyroid) malignancies in monkeys (as in humans) generally occur after a latency period of ten years or more[6]. After irradiation of the hypothalamus-pituitary axis a decreased secretion of hormones from the anterior lobe of the pituitary can occur. In most cases growth hormone is the first of these hormones to be decreased in the circulation. Radiation induced growth hormone deficiency (GHD) is dose dependent and the incidence increases with increasing post-irradiation intervals[27;28]. The animals in the high-dose group were therefore more likely to suffer from GHD than those in the low-dose group. However, the diagnosis of GHD is difficult and requires stimulation tests or evaluation of GH secretion patterns by frequent sampling, which could not be performed in the present study. Growth hormone deficiency is reflected by decreased serum levels of IGF-1 and IGFBP-3[29]. Even in man, however, the normal ranges of IGF-I and IGFBP-3 are wide and distinction between values of normal and GH-deficient subjects is difficult. Information on IGF-1 and IGFBP-3 levels in serum of normal rhesus monkeys is scarce, and values show considerable variations: Schwartz and Kemnitz[13] describe mean IGF-I levels of approximately 100-350 ng/ml in young adult rhesus monkeys, whereas Liu et al.[30] mention mean IGF-I levels of 600-1600 ng/ml. We therefore depended on our control animals as reference for normal values of IGF-I and IGFBP-3. Although the absolute levels of IGF-I and IGFBP-3 were not different between the radiation groups, the lower IGF-1/IGFBP-3 ratio of the animals in the high-dose group suggests less circulating free IGF-I, which may indicate subtle changes in the somatotrophic axis by TBI. Histological examination did not reveal any changes in the pituitary gland; whether the hyperplasia of prolactin-

producing cells is related to the TBI is unknown. In summary, we demonstrated an effect of TBI on body weight, ponderal index and skinfold thickness, but not on height. The histological changes and the decrease of thyroid weight with increasing TBI doses are indications for radiation-induced thyroid damage and compensatory reactions. Although the changes in body composition and the normal IGF-I and IGFBP-3 levels do not suggest radiation-induced GHD, there was a decreased IGF-I/IGFBP-3 ratio in the high-dose group, which could indicate a subtle effect of TBI on the somatotrophic axis. We therefore conclude that TBI-doses of 4-12 Gy with an instantaneous dose rate of 0.3 Gy min^{-1} can have an effect on the thyroid gland and on the physical build of primates even in the absence of cytostatic agents or immunosuppressive drugs.

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