PERGAMON

European Journal of Cancer 37 (2001) 1938–1945

European Journal of Cancer

www.ejconline.com

Extended-term effects of head and neck irradiation in a rodent

R.M. Nagler*

Department of Oral and Maxillofacial Surgery and Oral Biochemistry Laboratory, Rambam Medical Center and Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

Received 1 December 2000; received in revised form 20 April 2001; accepted 12 June 2001

Abstract

Radiotherapy to the head and neck is a common treatment for malignancies of the region. Unfortunately, exposure to irradiation often results in a variety of complications, most of which are localised and expressed in the short term following irradiation. However, prolonged and systemic effects may have greater clinical importance as the survival rate of head and neck irradiated patients is increasing yearly. Six groups of 18–20 rats were evaluated during a 1 year study. The non-irradiated control group was compared with 2.5 Gy, 5, 7.5, 10 and 15 Gy irradiated groups. We found a dose-dependent reduction in both survival and body weight in our rat models following a delayed, prolonged and chronic process. Dying animals were emaciated, dehydrated and starved, and many were blind and immunocompromised. While the exact underlying mechanism of this delayed, but devastating, phenomenon has not yet been determined, the delayed xerostomia inflicted on these animals may, at least partially, explain it. The clinical implications for head and neck patients require further evaluation, but our data should be considered, in the context of the available evidence for the long-term effects of head and neck irradiation in humans. © 2001 Published by Elsevier Science Ltd.

Keywords: Complications; Delayed; Prolonged; Xerostomia

1. Introduction

Irradiation (IR) delivered to the head and neck is a commonly used therapy for regional malignancies. The usual total IR dose is relatively high (30–80 Gy), often resulting in a variety of side-effects involving tissues and organs exposed within the IR field. The relatively widely studied complications are mainly regional, including xerostomia, osteoradionecrosis, swallowing disorders, carotid artery rupture, oropharyngeal mucositis or hypogeusia. Most of these regional complications are inflicted during the short-term post-IR [1–5]. Unfortunately, studies of late systemic complications due to head and neck IR are extremely scarce, although some persistent regional complications have been described, including sight and hearing impairment, accelerated stenosis of the carotid artery, glottic erythema and thyroid hypofunction [6–15]. This is a major omission, as the survival rate of head- and neck-irradiated patients is constantly increasing, and examining and treating such late effects may be of great clinical importance. In two recently published studies, we elaborated on the significant long-term salivary effects of head and neck IR in a rat model. Those studies further emphasise the clinical significance of the extended and prolonged effects of such irradiation. In this study, we chose to use the same rat model to evaluate the generalised systemic effects of head and neck IR [16,17]. Thus, the purpose of this study was to examine various systemic parameters during the first year following acute exposure of the rat head and neck region to irradiation. We chose the rat model as it is the most widely examined in the field, often used to evaluate localised head and neck IR effects such as induced xerostomia [18–29] or the induction of salivary gland tumours [30–32]. We also evaluated the validity of this animal model for studying human developmental abnormalities.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 250–300 g were purchased from Harlan-Sprague Dawley (Walkersville, MD, USA).

* Correspondence address: 17 Yaara Street, Timrat 23840, Israel. Tel.: +972-6-644-2003; fax: +972-6-654-1295.
E-mail address: nagler@tx.technion.ac.il (R.M. Nagler).

0959-8049/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0959-8049(01)00213-1

They were kept in polycarbonate cages under an alternating 12 h light:dark cycle. Animals were maintained on laboratory chow and water *ad libitum*. All experiments were performed in accordance with the Research Animal Care Committee of Rambam Medical Center and Faculty of Medicine.

2.2. Irradiation

Rats were anaesthetised by an intraperitoneal (i.p.) injection of sodium pentobarbital, 30 mg/kg (Somnifer, Richmond Veterinary Supply, Richmond, VA, USA), weighed and then placed in a box with a 3-mm lead shield, so that only the head and neck regions were exposed. The animals were irradiated with a single acute exposure of 2.5, 5, 7.5, 10 or 15 Gy delivered by two opposing 250 kV therapeutic X-ray tubes (Philips Medical System Inc.) operated at 235 kV, 15 mA, with an output of 1.26 Gy/min at 54 cm. All irradiation was carried out between 8:00 a.m. and 12:00 noon. Control animals were anaesthetised, but had no radiation delivered.

2.3. Body weight

Rats were housed three per cage until they reached 500 g of body weight; after that they were housed two per cage. All animals were weighed weekly until the age of 2 months. Then they were weighed on a monthly basis until the end of the study period.

2.4. Pathology, histology and organ harvesting

Rats were completely euthanised by CO_2 breathing and the following tissues were evaluated histologically after gross general pathological examination: brain, pituitary, tongue, oral and oesophageal mucosa, salivary glands, eyes, lachrymal glands, thymus, thyroid, maxillary and femoral bone marrow, cervical lymph nodes, trachea, lung, pancreas, kidney, duodenum, stomach, liver, heart and spleen. The last three organs and the epididymal fat (E. fat) were weighed as well. The evaluation was performed on control and 15 Gy groups at 2, 6 and 9 months post-IR.

2.5. Blood composition

Blood samples were collected via intracardial approach from rats lightly anaesthetised by breathing methophane at 1.5, 3 and 6 months post-IR for the control and 15 Gy irradiated groups. Blood composition was analysed by routine laboratory methods measuring the following parameters: haematocrit, complete blood count (CBC), differential, interleukin-6 (IL-6), total protein, glucose, triglycerides, uric acid, creatinine and the enzymes amylase and alkaline phosphatase.

2.6. Food replacement study

At 4 months post-15 Gy IR, the hard chow of four rats was replaced by powdered chow with the same nutritional content per weight unit. Three rats from the same group, equally irradiated, were maintained continuously on the hard chow, as were two controls from the same group of animals which were sham-irradiated. The body weight and food consumption of the nine animals were examined daily. Water and food were supplied *ad libitum* and the animals were housed separately, one per cage. The study was conducted for a month.

2.7. Taste preference study

Standard two-bottle preference tests were performed. At 12 months post-IR, four 7.5 Gy IR rats with a mean body weight of 637 g were compared with four similarly aged control rats with a mean body weight of 821 g. All testing used pairwise caged animals, each test consisting of presenting the animals with the opportunity to drink from a bottle containing distilled water and another bottle containing a test stimulus over a 7 h period. Preferences were measured, using several taste qualities: 0.3 M (sweet), 0.1 M NaCl (salty), 10 mM citric acid (acidic) and 1 mM quinine sulphate (bitter). Side preferences were avoided by switching the bottles three times during the first 15 min. After each test, the animals were deprived of water for 17 h and, between test stimuli, water consumption was measured for two test periods before commencing with a new stimulus. Preferences were calculated from two test periods for each stimulus and were expressed in mean percent preference over distilled water, a positive value indicating preference and a negative value indicating aversion. Additionally, taste buds containing papillae of the tongue were viewed with a light microscope to see if there were morphological differences between control and irradiated rats.

2.8. Statistical analysis

Significant differences of data evaluated were resolved with a paired Student's *t*-test. Values are expressed as means $\pm$ standard errors of the mean (S.E.M.); (*) and (**) denote $P<0.05$ and $P<0.01$, respectively.

3. Results

3.1. Survival

All the control 2.5 Gy (except for three animals who were sacrificed, as described below) and 5 Gy rats survived the entire 12 month study period. In contrast, in

the 7.5, 10 and 15 Gy groups there were delayed (later than 3 months post-IR) dose-associated animal deaths. In the 15 Gy group, 12/30 of the animals died during the acute post-IR phase (2 weeks) (Fig. 1) and thus 18 irradiated animals in the 15 Gy irradiated group were left for the rest of the study. Later on, 10 of these 18 rats died during the 3–9 month post-IR period and the remaining animals were sacrificed for examination purposes. In the 10 Gy group, 7/23 died during the 3–9 month post-IR period and another 5/16 during the 9–12 month post-IR period. In the 7.5 Gy group, 1/17 died during the 3–9 month post-IR period and another 3/16 during the 9–12 month post-IR period (Fig. 1). All deaths occurred following a prolonged period of emaciation (extensive fatty tissue wasting) and body weight loss, except for three 2.5 Gy animals who developed tumours for which they were sacrificed (discussed below).

3.2. Body weight

Until the 2–3 month period post-IR, there was continuous body weight gain for the control and irradiated animals, with one exception, a 15 Gy animal who actually lost weight during the first post-IR week and later regained it. At 2–3 months, the 15 Gy group and, at 6 months, the 10 Gy group started to lose weight. Starting from day 0, there was an IR dose–response effect on body weight gain of all irradiated animals (Fig. 2). Thus, at 9 months post-IR, the mean body weight of the control animals was 750±10 g, while that of the 7.5, 10 and 15 Gy groups were reduced by 21, 36 and 55%, respectively ($P<0.01$) (Table 1, Fig. 3). At 12 months post-IR, the mean body weights of the 7.5 and 10 Gy groups were reduced by 27 and 48%, respectively ($P<0.01$) (Fig. 2).

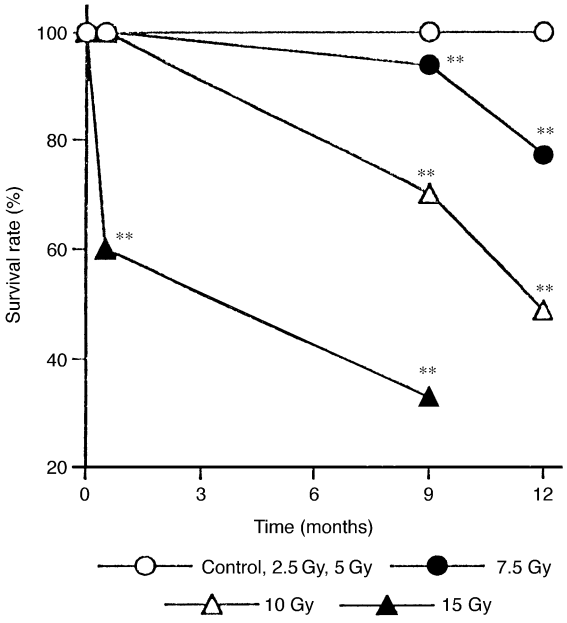

Fig. 1. The survival rate of irradiated [(IR)] versus control animals: by 2 weeks post-IR, only 15 Gy IR rats had died. By 9 months post-IR, the survival rates of 7.5, 10 and 15 Gy IR animals were significantly reduced ($P<0.01$) in comparison to the 2.5 and 5 Gy IR and control animals. By 12 months, no 15 Gy IR rats were available for analysis and 7.5 and 10 Gy IR rats had a lower survival rate than at 9 months.

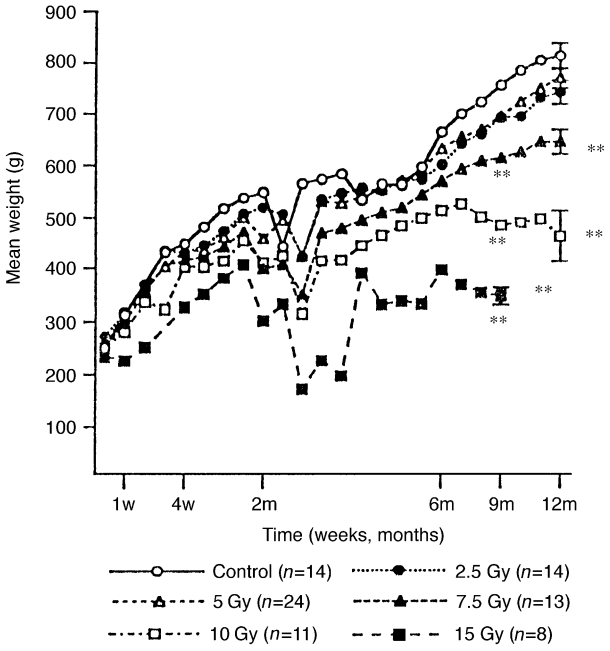

Fig. 2. Mean body weight of control and 2.5–15 Gy irradiated rats through a year of follow-up: the number of animals is noted in parentheses on the day the last measurement was obtained. **denotes a statistical significance of $P<0.01$ and is presented only for the 9 and 12 month measurements. w, weeks; m, months.

3.3. Organ weight

At 2, 6 and 9 months post-IR, the weights of the liver, heart, spleen and E. fat of control and 15 Gy rats were obtained (Table 1). The ratio between IR:control (% of control) values for the heart, spleen and total body

Fig. 3. Close distance photograph of control and 15 Gy irradiated rats at 9 months post-irradiation: the observed oral tubes were used to demonstrate the severely IR-induced salivary hypofunction, described elsewhere [16].

Table 1
The mean values of body and organ weights of 15 Gy irradiated versus control rats at 2, 6 and 9 months

	2 months		6 months		9 months	
	Control ($n=3$)	Irradiated ($n=3$)	Control ($n=4$)	Irradiated ($n=7$)	Control ($n=2$)	Irradiated ($n=3$)
Body weight (g)	538.0±33.0	423.6±22.9	661.6±32.5	309.1±31.5	750.0±10.0	335.0±25.6
Heart weight (g)	1.9±0.3	2.3±0.7	2.2±0.2	1.1±0.0	1.9±0.1	1.1±0.1
Spleen weight (g)	0.7±0.2	0.8±0.0	0.9±0.1	0.5±0.0	1.0±0.0	0.5±0.0
Liver weight (g)	21.5±0.9	17.3±1.7	25.8±3.1	11.0±0.8	31.7±0.3	9.6±0.1
Epididymal fat weight (g)	4.7±0.2	5.3±0.8	7.8±1.0	1.7±0.2	9.0±0.2	1.8±0.3

The most profound reductions were noted for epididymal fat and liver at 6 and 9 months. The weight reductions of these tissues overrode the amount of body weight reduction, unlike the reductions in spleen and heart weights which were similar to that of the body weight reductions.

weights at 2, 6 and 9 months and for liver and E. fat at 2 months were similar (Fig. 4). However, at the later time points, 6 and 9 months, the ratios for liver, and even more so for E. fat, dropped to lower values in comparison with those for total body, heart and spleen weights. Thus, the IR:control ratio values at 6 and 9 months for the liver were 0.42 and 0.30, respectively, and for the E. fat 0.21 and 0.20, respectively (Fig. 4).

3.4. Pathology

The following changes were seen in the irradiated rats: parotid and submandibular atrophy, lymphocyte infiltration and acinar nuclear hypertrophy of lachrymal glands, cataracts, femoral and mandibular bone marrow hypoplasia, liver and thymus atrophy, dermatitis of the head and neck region, emaciation, incisor malocclusion,

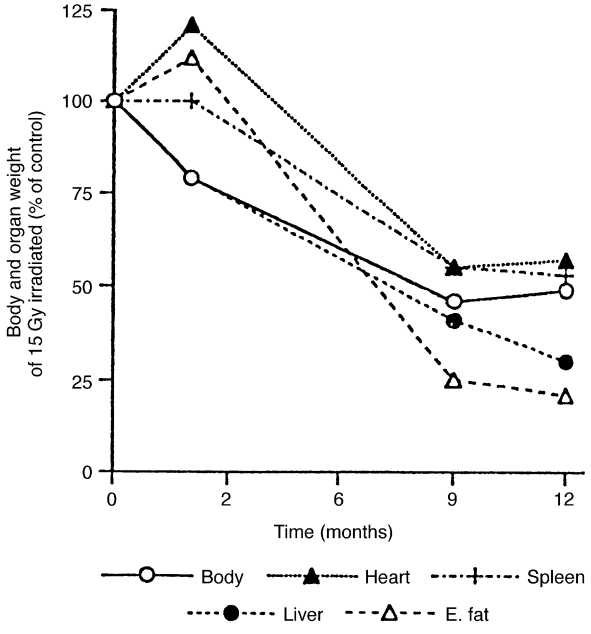

Fig. 4. Values of the ratio between mean body and organ weights of 15 Gy irradiated and control animals at 2, 6 and 9 months: calculations were made according to the results presented in Table 1. Values for E. fat at 9 and 12 months and liver at 12 months post-IR were significantly lower ($P<0.01$) than those of body weight, indicating the major tissue targets involved.

tongue ulcers and neoplasms. In contrast, no histopathological differences could be found in the examined pituitary, brain or thyroid tissues of irradiated rats. Splenic haemosiderosis was found in both the irradiated and control rats, and we assume that it is an age-related change and not related to IR; its incidence increased in both populations by age.

- Parotid gland atrophy was found in the 9 month 2.5 Gy rat (1/1 examined), in the 6 month 15 Gy rat (4/4 examined), and in the 11 day 15 Gy rat in which it was probably unrelated to the IR because the collagen appeared mature. Thus, parotid gland atrophy was observable after 6 months following higher doses of IR.
- Submandibular gland atrophy was found in the 2 month 15 Gy (1/2), 6 month 10 Gy (1/1), 9 month 7.5 Gy (1/1) and 10 Gy (1/1) rats. Submandibular gland atrophy was observed routinely after 6 months following a 2.5 Gy and higher dose.
- Lachrymal gland: Lymphocytic infiltrates were found in a 6 month 15 Gy rat, but not in the nine month 2.5 Gy or 11 day 15 Gy rats. Nuclear hypertrophy (increased variation in nuclear size of the acinar cells) with some hypertrophied nuclei (2–3×normal size) was found in the 9 month 2.5 and 7.5 Gy rats, but not in the 11 day 15 Gy or the 6 month 15 Gy rats. Thus, lymphocytic infiltration of the lachrymal gland was induced by a high dose (15 Gy) and nuclear hypertrophy by a low dose of IR, and both after a long latent period (over 6 months).
- Cataracts were found to be dose-related following a long period and high doses of IR. At 12 months, 9/13 7.5 Gy rats (69%) and 9/11 10 Gy rats (81%) had cataracts (Fig. 5). In contrast, cataracts were not found in 11 day 15 Gy or 9 month 2.5 Gy rats.
- Bone marrow hypoplasia was found in the femor of 6 month 15 Gy rats (2/3 examined) and one 9 month 7.5 Gy rat. Bone marrow hypoplasia was also found in the mandible in three rats, one 11 day 15 Gy (1/5 examined) and two 9 month 15 Gy

Fig. 5. 15 Gy irradiated rat at 9 months post-irradiation: the inflicted reduction in body size and bilateral cataracts are clearly noted.

(2/3 examined) rats; this seems to be directly IR-induced.

- Liver and thymus atrophy: Liver atrophy was found in five animals: 6 month 10 Gy (1/1 examined), 15 Gy (2/7 examined), nine month 2.5 Gy (1/2 examined), 7.5 Gy (1/1 examined). Histologically, hepatocytes were 0.5–0.8 the size of control hepatocytes due to the reduced volume of cytoplasm, always occurred in emaciated animals, and were probably secondary to the reduced volume. Thymus atrophy was found in 15/19 of the examined irradiated rats.
- Malocclusion (overeruption of unground anterior incisor teeth) was found only in long-term animals (6–9 months) for all IR doses and always resulted in emaciation. Three of four animals examined also had submandibular atrophy, while 4/4 had thymic atrophy.
- Skin dermatitis was found in the head and neck region of all the 11 day 15 Gy rats examined. Histologically, the lesion is compatible with IR-induced dermatitis.
- Tongue ulcers were found in 6/9 animals at 11 days after 15 Gy, but not in long-term irradiated animals. All the animals with tongue ulcers were emaciated.
- Emaciation was found in 10/13 examined animals: 11 day 15 Gy (5/5 examined), 6 month 10 Gy (1/1 examined) and 15 Gy (1/2 examined), 9 month 2.5 Gy (1/2 examined),10 Gy (1/1 examined) and 7.5 Gy (1/2 examined). Emaciated animals had the following other changes: parotid gland atrophy (1/8), submandibular gland atrophy (3/9), lachrymal gland inflammation (0/3), lachrymal gland nuclear hypertrophy (2/3), cataracts (3/6), femur bone marrow hypoplasia (2/6), mandible bone marrow hypoplasia (2/9), thymic atrophy (9/9), liver atrophy (4/10), dermatitis (5/10), malocclusion (5/10) and tongue ulcers (3/10).
- Neoplasms: Three animals developed neoplasms at a late post-IR time phase: one 6 month 2.5 Gy rat had a poorly differentiated solid carcinoma of salivary gland origin, one 6 month 2.5 Gy had malignant schwannoma appearing in the salivary gland, and one 9 month 2.5 Gy rat had dermal fibroma. None of these animals was emaciated or had malocclusion. No animal irradiated with a dose higher than 2.5 Gy developed neoplasms. Irradiation-induced salivary neoplasms are not surprising, but why this should occur only after administration of the lowest dose (2.5 Gy) is puzzling.

3.5. Blood composition

At 1.5 and 3 months, there were no significant differences between IR and control animals for any of the examined parameters. No differences could be found at six months except for WBC, lymphocytes and triglycerides. The mean values of WBC, lymphocytes and triglycerides were reduced in the 15 Gy group by 36% ($P<0.01$), 46% ($P<0.01$) and 49% ($P<0.05$), respectively (Table 2).

3.6. Food replacement study

The IR rats who were maintained on hard chow lost 0.6 g±0.4 of body weight and consumed 14.1 g±0.7 of food per day. The sham-irradiated control animals gained 1.1 g±0.2 of body weight and consumed 27.7 g±0.1 of food per day. The IR rats which had powdered chow instead of the hard chow gained 0.2 g±0.1 of body weight per day, showing a large diversity among the animals which consumed an average of 32.6 g±3.4 of food per day. The food consumption of the IR-powdered chow and control groups were not significantly different but were significantly larger than the IR-hard chow group. Yet, the body weight gains of

Table 2
The mean values of white blood cells, lymphocytes and triglycerides in the sera of rats at 6 months post-irradiation (15 Gy)

	White blood cells (10^3/μl)	Lymphocytes (10^3/μl)	Triglycerides (100 mg/l)
Controls ($n=8$)	8.8±0.8	8.3±0.8	15.6±3.1
Irradiated (15 Gy) ($n=14$)	5.6±0.3**	4.5±0.2**	8.0±1.0*

Values significantly reduced are denoted by: *$P<0.05$; **$P<0.01$.

Table 3
Mean values of body weight gain and food intake of irradiated animals versus controls at 4 months post-15 Gy irradiation

	Body weight gain [g/day]	Food intake [g/day]
Controls ($n=2$)	1.1 ± 0.2	27.7 ± 0.1
Irradiated hard chow ($n=3$)	-0.6 ± 0.4	14.1 ± 0.7
Powder chow ($n=4$)	0.2 ± 0.1	32.6 ± 3.4

The food composition of irradiated power chow and control animals were similar but significantly greater ($P<0.01$) than that of irradiated hard chow animals. However, the body weight gains of both irradiated groups were similar and significantly lower than that of controls ($P<0.01$).

both IR groups, while not significantly different, were significantly lower than those of controls (Table 3).

3.7. Taste preference study

At 12 months post-IR, both 7.5 Gy irradiated and control rats showed a marked preference for sucrose, an aversion to quinine and citric acid, and no preference for or aversion to NaCl. There was a quadrupling of fluid consumption when sucrose was included in the test, compared with normal water tests. In tests using citric acid and quinine, there was normal fluid consumption with nearly all the fluid being taken from the water bottle. The results for NaCl were less clear with an almost equal consumption of NaCl and water within the error range of this two-bottle test. The tests show no noticeable long-term effects to the ingestive behaviour of rats following high level irradiation (7.5 Gy).

The light microscope examination of the tongue surface showed that the circumvallate, foliate and fungiform papillae were morphologically alike in control and irradiated rats, and that the number of these papillae were equal in the control and irradiated rats.

4. Discussion

Irradiation of the rat head and neck region resulted in devastating systemic effects. During the short-term post-IR (first 2 weeks), they are considered as resulting from a severe crisis proceeding from oropharyngeal mucositis induced by the IR, as previously reported [24,31,32]. Our short-term results demonstrating a dose-dependent reduction in body weight gain in the 2.5–15 Gy animals, accompanied by oral mucositis, skin dermatitis, tongue ulcers, emaciation and death of 40% of 15 Gy IR animals are in agreement with previous reports. However, the present study focuses on the delayed effects of head and neck IR, as these effects were rarely evaluated in the past in spite of their significant clinical importance, particularly now that the survival rate of head and neck irradiated patients is higher than ever before.

At a later post-IR phase (up to 2–3 months) following the short-term crisis, the observed animals recovered, although the body weight gain of all groups, including the surviving 15 Gy rats, was reduced in a dose-dependent manner. During the recovery phase, no animal deaths occurred and no IR effects on organ weight, pathology or blood composition were demonstrated. However, when the recovery phase came to an end at approximately 3 months post-IR, a devastating and prolonged process started, also in a dose-responsive manner. At 3 and 6 months, the 15 and 10 Gy groups respectively animals began to lose weight. At nine months post-IR, the decrease in body weight for the 7.5, 10 and 15 Gy rats was by 21, 36 and 55% ($P<0.01$), respectively. During this late deterioration phase, the animals also suffered from a wide spectrum of other injuries which, at nine months, resulted in death rates of 6, 30 and 55% in the 7.5, 10 and 15 Gy rats, respectively.

Delayed IR-induced effects in rats were reported for the first time in 1957 [20], but were never studied in detail and the underlying mechanisms remained obscure. A few reports of retardation in the growth and development of children exposed to head and neck IR are available [5,7,11], although it is not clear whether there is any association between the human and animal phenomena.

Our results for rats resemble somewhat those reported in 1967 by Mosier and Jansons [10] with respect to the late reduction in rat body weight following head and neck IR. However, the phenomenon we describe has more chronic characteristics and is far more prolonged. Mosier noted that the survival rate of irradiated animals decreased to 0% at 6 months post-13–14 Gy IR, accompanied by both anaemia and septicaemia while, in our case, 45% of the 15 Gy rats survived until they were sacrificed at 9 months with no changes in the erythrocyte counts. The difference between our results and those of Mosier and Jansons could be attributed to the different rat model or mode of irradiation used.

We demonstrated no histological effect of IR on the brain, pituitary or thyroid, in accordance with the previously reported inability to prevent head and neck IR-induced growth disturbance with either growth hormone or thyroxin [11,13]. Thus, it is improbable that IR effects on brain, pituitary or thyroid are the cause of the phenomenon. Some of the late effects seem to be directly induced by IR and involve organs within the radiation field, such as parotid and submandibular atrophy, mandibular bone marrow hypoplasia, lachrymal gland lymphocytic infiltration with cellular aberrations and cataracts. With respect to cataracts, there is profound similarity between our animal results and human reports; it has been reported [11] that no less than 80% of head and neck patients irradiated with 10 Gy developed cataracts within 6 weeks, while we noted

cataract rates of 68 and 82%, respectively, in the 7.5 Gy and 10 Gy animals at 12 months post-IR.

In contrast to these few direct effects, however, other effects seem to be secondary to a delayed IR-induced inanition. Thus, the animals suffered from a prolonged dose-related reduction of their food intake which resulted in overgrowth of their unground anterior incisor teeth (malocclusion), which further reduced the animal's ability to consume food. Secondary to this, the animals suffered from emaciation as well as severe reduction in fat tissue, serum concentration of triglycerides and liver atrophy. Furthermore, the animals suffered from reduced levels of white blood cells and lymphocytes in their serum and, in some high-dose irradiated animals (7.5–15 Gy), also from femoral bone marrow hypoplasia. It is noteworthy that a recent paper reported the development of functional impairment of granulocytes and lymphocytes 1 year following IR to the head and neck region of 9 patients [12]. We assume that the concomitant effect of the bone marrow hypoplasia, thymus atrophy and reduction in white blood cells and lymphocytes in the sera resulted in a severely compromised immunoresistance. It may even be presumed that this immunosuppression is partially secondary to the inflicted malnutrition, as such an association may occur [14]. Thus, the combination of inanition and immunodeficiency could explain the prolonged deterioration of the animals and their eventual death.

In order to try to further elicit the mechanism causing the dose-dependent reduction in food intake, we examined a hypothesis suggesting that the animals had aberrations in their lingual papillae or taste, but we failed to demonstrate this. Another hypothesis raised was that a profound reduction in salivary function, a known aetiology for dysphagia [9,13,15], was the cause of the delayed food intake reduction. In the present study, both parotid and submandibular atrophy were found at six months and later following IR. In previous studies, we demonstrated a dose-dependent reduction of salivary function of both parotid and submandibular glands, statistically significant for 7.5 Gy IR doses and higher, at 40 days post-IR [19]. In other studies, a post-IR extensive and continuous reduction in the function of the salivary glands, accompanied by a concomitant quantitative change in salivary composition, throughout the year of study was demonstrated [22,26]; such hyposalivation could contribute, at least partially, to the observed chronic and eventually lethal dysphagia, as hyposalivation is a well-known aetiology of dysphagia. The significant increase in the food intake of the irradiated animals when their hard chow was replaced by powder chow adds support to this hypothesis. Powder chow is easier to swallow by xerostomic animals and such a replacement helps the animals to overcome the induced dysphagia [9,13,15]. Further support may be indicated by the similar kinetics of both deterioration of salivary function [20] and the dysphagia-induced overeruption of the ungrinded anterior teeth. Both phenomena developed at a delayed phase and both occurred for all irradiated animals. At 12 months post-IR, even following 2.5 Gy only, significant reductions of both parotid and submandibular functions were demonstrated [22].

In summary, the accumulated data demonstrate the devastating outcome of head and neck irradiation in a rat model. The effect of the irradiation is dose-dependent and is manifested by a prolonged and chronic deterioration in the general status, eventually resulting in the animal's death. The exact mechanism underlying this delayed phenomenon, in which the two major characteristics are dysphagia and an immunodeficiency state, is unknown at present. It seems that the severe IR-induced delayed salivary hypofunction may play a significant role in this mechanism. The bone marrow hypoplasia induced by the head and neck irradiation, which has never been described before, may also be attributed to the long-term starvation of the animals. Future studies aimed at elucidating this mechanism and evaluating the clinical importance of these results in humans are warranted.

References

1. Kashima HK, Kirkham WR, Andrews JR. Post-irradiation sialadenitis: a study of the clinical features, histopathologic changes and serum enzyme variations following irradiation of human salivary glands. *Amer J Roentgenol Rad Ther Nucl Med* 1965, **94**, 271–291.
2. Koka VN, Deo R, Lusinchi A, *et al*. Osteoradionecrosis of the mandible: study of 104 cases treated by hemimandibulectomy. *J Laryngol Otol* 1990, **104**, 305–307.
3. Lazarus CL, Logemann JA, Pauloski BR, *et al*. Swallowing disorders in head and neck cancer patients treated with radiotherapy and adjuvant chemotherapy. *Laryngoscope* 1996, **106**, 1157–1166.
4. Talmi YP, Finkelstein Y, Zohar Y. Post-irradiation hearing loss. *Audiology* 1989, **28**, 121–126.
5. Cooper JS, Fu K, Marks J, Silverman S. Late effects of radiation therapy in the head and neck region. *Int J Radiat Oncol Biol Phys* 1995, **31**, 1141–1164.
6. Appleton RE, Farrell K, Zaide J, Rogers P. Decline in head growth and cognitive impairment in survivors of acute lymphoblastic leukemia. *Arch Dis Child* 1990, **65**, 530–534.
7. Griffin NK, Wadsworth J. Effect of treatment of malignant disease on growth in children. *Arch Dis Child* 1980, **55**, 600–603.
8. Maisin J, Maldague P, Dunjic A, Maisin H. Syndromes mortels et effets tardifs des irradiations totales et subtotales chez le rat. *J Belg Radiol* 1957, **40**, 346–398.
9. Mandel ID. The role of saliva in maintaining oral homeostasis. *J Amer Dent Assoc* 1989, **119**, 298–304.
10. Mosier Jr. HD, Jansons RA. Stunted growth in rats following x-irradiation of the head. *Growth* 1967, **31**, 139–148.
11. Saunders JE. Implications of cancer therapy to the head and neck on growth and development and other delayed effects. *Nat Cancer Inst Monogr* 1990, **9**, 163–167.
12. Spiechowicz E, Rusiniak-Kubik K, Skopinska-Rozewska E, *et al*. Immunological status of patients with denture stomatitis and

yeast infection after treatment of maxillofacial tumors. *Arch Immunol Ther Experiment* 1994, **42**, 263–267.
13. Sreebny LM. Recognition and treatment of salivary induced conditions. *Int Dent J* 1989, **39**, 197–204.
14. Suskind RJ. *Malnutrition and the immune response*. New York, Raven Press, 1977.
15. Cooper JS, Fu K, Marks J, Silverman S. Late effects of radiation therapy in the head and neck region. *Int J Radiat Oncol Biol Phys* 1995, **31**, 1141–1164.
16. Nagler RM, Baum BJ, Miller G, Fox PC. Long-term salivary effects of single-dose head and neck irradiation in the rat. *Arch Oral Biol* 1998, **43**, 297–303.
17. Nagler RM. Short- and long-term functional vs morphometrical salivary effects of irradiation in a rodent model. *Anticancer Res* 1998, **18**, 315–320.
18. Hiramatsu Y, Nagler RM, Baum BJ, Fox PC. Rat salivary gland blood flow and blood-to-tissue partition co-efficients following x-irradiation. *Arch Oral Biol* 1994, **39**, 77–80.
19. O'Connell AC, Redman RS, Evans RL, Ambudkar IS. Radiation-induced progressive decrease in fluid secretion in rat submandibular glands is related to decreased acinar volume and not impaired calcium signaling. *Radiat Res* 1999, **151**, 150–158.
20. Nagler RM, Baum BJ, Fox PC. Acute effects of x-irradiation on the function of rat salivary glands. *Radiat Res* 1993, **136**, 42–47.
21. Nagler RM, Baum BJ, Fox PC. Effects of x-irradiation on the function of rat salivary glands at 3 and 40 days. *Radiat Res* 1993, **136**, 392–396.
22. Nagler RM, Baum BJ, Fox PC. Long term effects of x-irradiation on rat salivary gland. *J Dent Res* 1994, **73**, 213 (abstr.).
23. Nagler RM, Baum BJ, Fox PC. A 2 week pair-fed study of early x-irradiation effects on rat major salivary gland function. *Arch Oral Biol* 1996, **41**, 713.
24. Nagler RM, Nagler A. Effects of ionizing irradiation and β-adrenergic stimulation on gene expression in rat submandibular glands. *Anticancer Res* 1996, **16**, 2749–2756.
25. Nagler RM, Marmary Y, Fox PC, *et al*. Irradiation induced damage to the salivary glands: the role of redox-active iron and copper. *Radiat Res* 1997, **147**, 468–475.
26. Nagler RM, Nagler A, Laufer D. Sialochemical profile of x-irradiated major salivary glands: an extended term animal study. *Int J Radiat Biol* 1997, **71**, 441–448.
27. Coppes RP, Zeilstra LJ, Vissink A, Konings AW. Sialogogue-related radioprotection of salivary gland function: the degranulation concept revised. *Radiat Res* 1997, **148**, 240–247.
28. Glucksman A, Cherry CP. The induction adenomas by the irradiation of salivary glands of rats. *Radiat Res* 1962, **17**, 186–202.
29. Ron E. Ionizing radiation and cancer risk: evidence from epidemiology. *Radiat Res* 1998, **150**, S30–S41.
30. Land CE, Saku T, Hayashi Y, *et al*. Incidence of salivary gland tumors among atomic bomb survivors. *Radiat Res* 1996, **46**, 28–36.
31. Menard TW, Izutsu KT, Ensign WY, *et al*. Radioprotection by WR-2721 of γ-irradiated rat parotid gland: effect on gland weight and secretion at 8–10 days post-radiation. *Int J Radiat Oncol Biol Phys* 1984, **10**, 1555–1559.
32. Quastler H, Austin MK, Miller M. Oral radiation death. *Radiat Res* 1956, **5**, 338–353.