Evaluation of Insulin-like Growth Factor-1 in a Mouse Model of Long-term Abdominal Radiation Toxicity

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Abstract. *The purpose of these experiments was to test whether a brief course of insulin-like growth factor-1 (IGF-1) injection (escalating doses) concomitant to irradiation ameliorates radiation-induced kidney dysfunction and lethal bowel toxicity in a mouse model of unilateral high-dose irradiation of the kidney and adjacent bowel. The kidney function was assessed by means of repeated ^{99m}Tc-dimercaptosuccinate scans (every six weeks) during a maximum follow-up of 49 weeks. The experiments with single fractions of 12 Gy and 15 Gy revealed only minor differences in the severity of kidney dysfunction and no reduction in lethal bowel toxicity from IGF-1 treatment. In the absence of any significant radioprotective effect, other strategies of response modification need to be developed.*

When treating cancer patients with ionizing radiation, the ultimate aim is to achieve high tumor control rates and few normal tissue complications. Despite intense research efforts over the last decades, no pharmacological toxicity prevention strategy for kidney irradiation was effective enough to enter the clinical practice (6, 14). Recent developments, such as intensity-modulated radiotherapy have improved our ability to shape the dose distribution to the target volume and, thus, spare more of the normal tissues. Despite all these advances, the kidney still represents a major dose limiting abdominal organ, preventing the application of a high radiation dose on some occasions.

Several groups have studied the emerging role of growth factors as mediators and modifiers of radiation reactions in normal tissues (1-5). Their results in various models suggest that both acute and late effects can be modulated by administration of, for example, keratinocyte growth factor, platelet-derived growth factor, erythropoietin (EPO) and insulin-like growth factor-1 (IGF-1). Our group has recently established a mouse model of radiation-induced kidney dysfunction (1). Initial experiments revealed that EPO did not protect the kidney and that the model is also suitable for assessment of lethal bowel toxicity. The latter finding was an accidental one and relates to technical aspects of radiation administration, which will be discussed later.

In the kidney, a variety of changes in different cell types occur after irradiation, which have been comprehensively reviewed by Cohen and Robbins (6). Changes include increased vascular permeability and perfusion disturbance (7, 8). IGF-1 receptors are present in various cell types in the kidney and their ligand IGF-1 increases both renal blood flow and glomerular filtration rate and reduces renal vascular resistance (9, 10), possibly resulting in improved kidney function. Encouraging data have also been reported with regard to intestinal radioprotection (11-13). Therefore, the effects of IGF-1 and the dose-response relation in our established mouse model have been investigated.

Materials and Methods

Animals. Female adult C3H/N mice (12-weeks-old and weighing approximately 22-25 gr) were purchased from Charles River Laboratories, Sulzfeld, Germany. Animals were housed under controlled conditions in conventional rodent facilities at the Department of Experimental Oncology, Klinikum rechts der Isar, Munich, Germany (20 mice per cage, access to water and commercially available rodent diet *ad libitum*). These facilities are accredited by the government of Upper-Bavaria and are operated in accordance with state standards and laws. The Governmental Animal Care and Use Committee approved the experimental protocol.

Irradiation and anesthesia. Mice were treated in a prone position with 70 kV X-rays (Philips RT100, Eindhoven, The Netherlands) to a 1.5-cm wide single field that included the right kidney, and also

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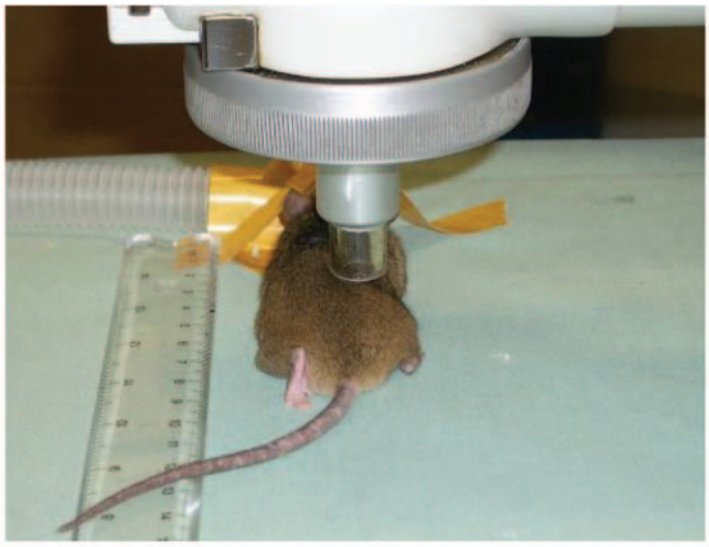

Figure 1. *Irradiation set up in a mouse anaesthetized by isoflurane inhalation.*

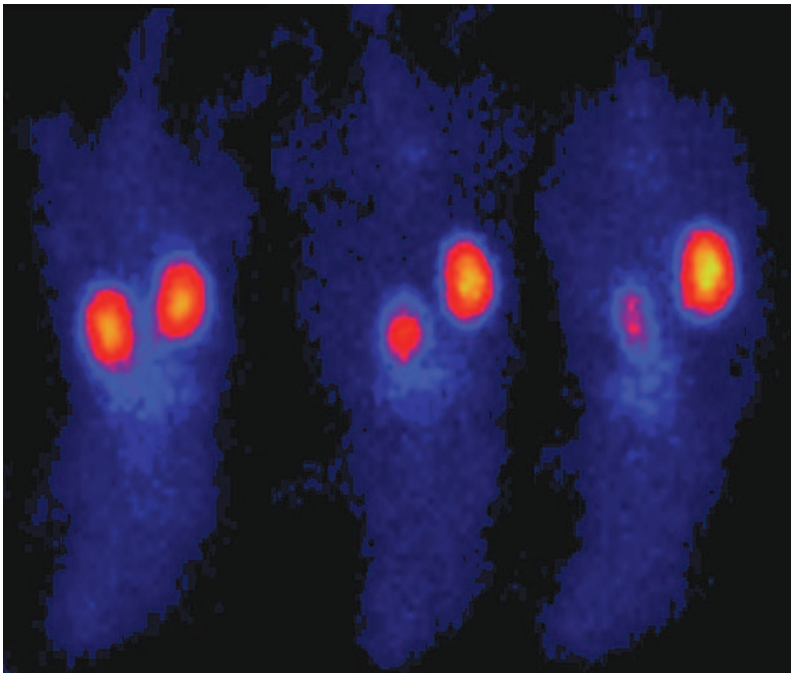

Figure 2. *Static scintigraphy before irradiation (left panel), after 37 weeks of follow-up (middle), and after 43 weeks (right). Typical example of severe progressive kidney dysfunction.*

adjacent bowel (at a dose rate of approximately 6 Gy per minute and source skin distance of 10 cm, Figure 1). The dose was prescribed to the surface of the kidney based on dosimetric measurements in cadavers performed by a medical physicist. The detailed set-up and dose distribution have been described previously (1). Radiation was administered in single fractions of 12 or 15 Gy, based on our previous experience of only minor changes in kidney function with 6-10 Gy (1). The animals were anesthetized during irradiation by inhalation of 1.5-2.0% halothane (plus oxygen 0.5 l/min) using a semi-circuit inhalation anaesthesia system to immobilize them in the desired position. The initial group size was at least six mice. Depending on the number of intercurrent deaths, additional animals were treated and added.

Assessment of response. The kidney function was assessed prior to radiotherapy, as well as 19 weeks thereafter and then every six

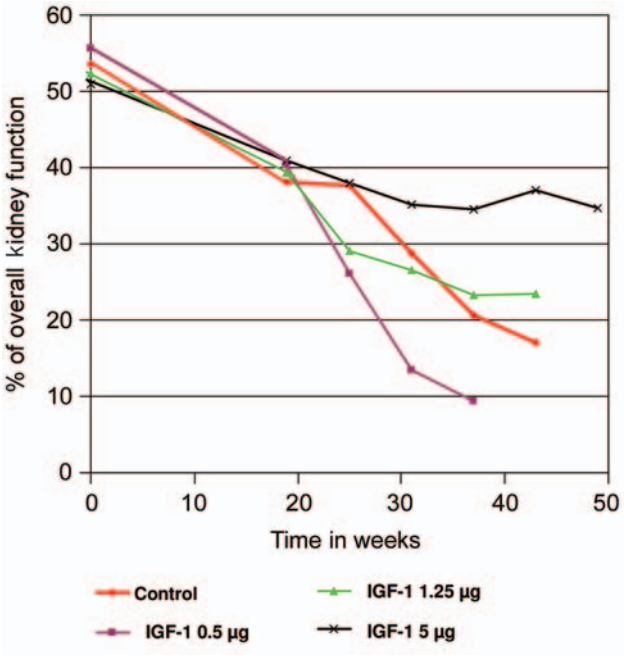

Figure 3. *Time course of the median contribution of the irradiated right kidney (12 Gy) to overall kidney function in % with or without IGF-1. The differences are not statistically significant.*

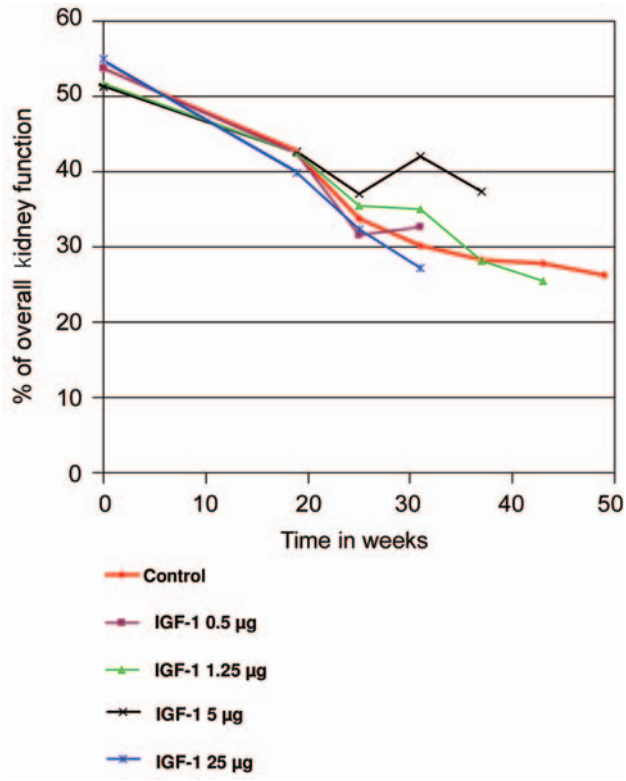

Figure 4. *Time course of the median contribution of the irradiated right kidney (15 Gy) to overall kidney function in % with or without IGF-1. The differences are not statistically significant.*

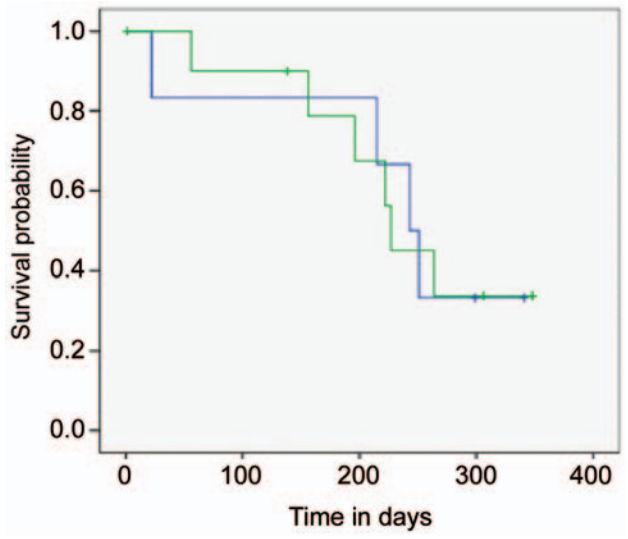

Figure 5. *Kaplan-Meier estimates of overall survival after irradiation with 12 Gy with (green line) or without (blue line) IGF-1 (5 µg per injection), median 236 vs. 243 days (p=0.96). Mice dying from anaesthesia complications are censored.*

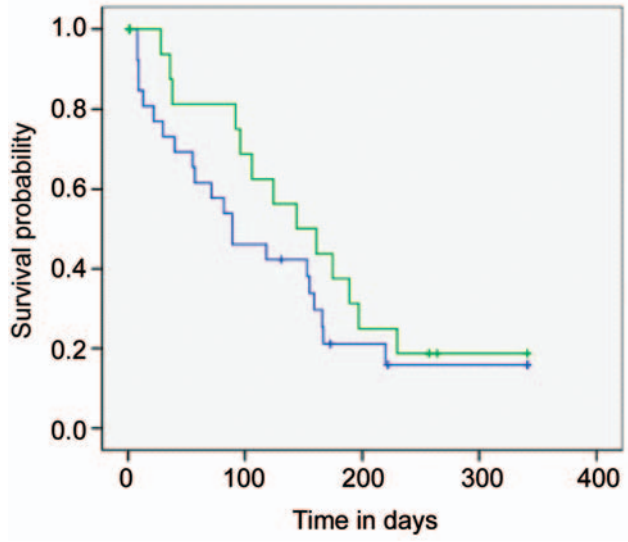

Figure 6. *Kaplan-Meier estimates of overall survival after irradiation with 15 Gy with (green line) or without (blue line) IGF-1 (5 µg per injection), median 127 vs. 165 days (p=0.28). Mice dying from anaesthesia complications are censored.*

weeks for a maximum of 49 weeks by means of ^{99m}Tc-dimercaptosuccinat (DMSA) scans in the Department of Nuclear Medicine, Klinikum rechts der Isar der Technischen Universität München, Germany. As previously outlined, this static scintigraphic method acquires relative measurements of the kidney function without absolute quantitative information (1). However, this method does allow for examination of the ipsilateral function after irradiation, which is expected to decline steadily from

Table I. *Overview of animal groups in the IGF-1 dose-finding experiment.*

IGF-1 dose	Radiation dose	n (death from anaesthesia)	Initial function[a]	Time of toxicity-related death, days	*n* alive at 4th scintigraphy[b]
No IGF-1	15 Gy	24 (3)	54±2%, 54	8, 8, 9, 9, 13, 22, 30, 40, 55, 57, 71, 82, 89, 89, 118, 153, 155, 159, 166, 167, 220	2
0.5 μg	15 Gy	10 (1)	54±4%, 54	19, 61, 79, 214, 215, 215, 215, 224, 232	2
1.25 μg	15 Gy	13	53±3%, 52	13, 28, 98, 105, 126, 171, 217, 221, 227, 316	7
5 μg	15 Gy	11	50±4%, 50	28, 36, 38, 92, 96, 106, 124, 144, 161, 189, 264	1
25 μg	15 Gy	6	55±3%, 55	17, 20, 184, 185, 215, 218	1
No IGF-1	12 Gy	6 (1)	53±2%, 54	22, 215, 243, 251	4
0.5 μg	12 Gy	10 (2)	55±6%, 56	27, 131, 168, 230, 250, 258, 258, 258	5
1.25 μg	12 Gy	10	53±3%, 53	24, 28, 191, 201, 301	6
5 μg	12 Gy	10 (2)	51±2%, 51	56, 156, 196, 222, 227, 264	6

[a]Contribution of the right kidney to the overall kidney function (mean, standard deviation, median); [b]31 weeks after irradiation.

approximately 50% of the total function in healthy mice at baseline. The reproducibility of the scintigraphic measurements is in the order of 3% (1). An autopsy was performed for all mice that died during follow-up to confirm intestinal radiation toxicity. Statistical evaluation of the kidney function (Mann-Whitney and Wilcoxon tests) and overall survival (Kaplan-Meier estimates and log-rank test) was performed by use of the SPSS software (SPSS, Chicago, USA). For survival analysis, mice dying from anaesthesia complications were censored at the time of death. A p-value of <0.05 was considered statistically significant.

IGF-1 injection. Groups of at least six mice received subcutaneous injections of rh-IGF-1 (Genentech, Inc., South San Francisco, USA) from 0.5 μg to 25 μg per dose 16 hours and 2 hours before radiation treatment and 20 hours thereafter. Table I summarizes all groups that form the basis of this experiment.

Results

Our assumption that radiation doses of 12 Gy and 15 Gy would induce significant kidney dysfunction in control mice was valid. Before irradiation, the right kidney consistently contributed 50-55% of the total kidney function (median values of all groups, median of all animals combined: 53%). After 25 weeks, the kidney function had declined significantly. Figure 2 shows an example of continuous deterioration over time. The lowest dose of IGF-1 (0.5 μg per injection) showed no protective effect. Treatment with 5 μg IGF-1 per injection appeared to slightly reduce the median loss of function (Figures 3 and 4). The highest dose of 25 μg per injection, which was only tested with 15 Gy, clearly failed to prevent renal insufficiency. None of the differences between the groups reached statistical significance, because the number of mice dying from intestinal toxicity, confirmed by autopsy, was unexpectedly high (Table I). After 12 Gy, 23 out of 36 mice (64%) died after a median of 215 days. After the higher dose of 15 Gy, 57 out of 64 mice (89%) died after a shorter median latency of 106 days. In addition, nine mice died from anaesthesia complications during follow-up scintigraphy. Other reasons for intercurrent death were not found. Due to this high drop-out rate, the statistical power for long-term evaluation of kidney function was not sufficient. None of the IGF-1 dose levels improved survival to a meaningful extent; examples are shown in Figures 5 and 6.

Discussion

Our mouse model is based on static scintigraphy without the need for blood drawing and laboratory analysis. Repeat analysis of a group of control mice has shown a high reproducibility of the scintigraphic measurements (1). To account for set-up and organ motion uncertainties, the radiation portal is larger (15 mm diameter) than the maximum kidney size (8-10 mm). Therefore, the full prescription dose is also being delivered to parts of the intestine. As 10 Gy caused occasional cases of lethal bowel complications in our previous study, account was taken of the higher toxicity of 12 or 15 Gy. However, due to encouraging reports of intestinal radioprotection with IGF-1, extended animal survival was hoped for to allow long-term assessment of the kidney function. The range of IGF-1 doses and timing of administration was chosen from experiments in different models of central nervous system and bowel damage. It has been reported that a single subcutaneous injection provides adequate plasma levels for 24 h (15). These data guided our choice of administration schedule.

Surprisingly, the death rates were extraordinarily high and there was no indication of a radioprotective effect of IGF-1 with regard to the intestine. Consistent with radiobiological principles, the death rate was higher after 15 Gy and the latency time to lethal intestinal complications was shorter. Initially more mice were added to the 15 Gy control group. However, when it became evident that long-term follow-up would not be possible, the decision was

taken to proceed with the smaller groups of surviving mice. None of the previous bowel studies has focused on long-term survival. Their endpoints were apoptosis, histological damage and other early changes in rodents (11-13, 16). However, the range of both radiation and IGF-1 doses was largely comparable with the present study and therefore, the connection between early assessments and long-term bowel damage requires further investigation.

Regarding the kidney function, limited follow-up and statistical power precluded any significant result. At first sight, a radiation dose of 12 Gy appeared to cause more damage than a dose of 15 Gy (Figures 3 and 4). However, this is most likely to be artefact due to the early deaths especially of the most severely damaged mice in the 15 Gy group. With an IGF-1 dose of 5 μg per injection, a slight improvement in the preservation of the kidney function might be possible. Potential mechanisms of action of IGF-1 at the cellular or molecular level include inhibition of apoptosis (13), cell cycle arrest at the more radioresistant G1 phase (17), and DNA repair (18). Different time frames of IGF-1 administration have not been examined. It is therefore unknown whether prolonged treatment would improve the results. Long-term application of 1.2 mg/kg/day for six weeks has been tolerated without complications in mice (19). Histological examinations of the major organs excluded significant side-effects. In future studies, fractionated radiotherapy should be preferred, because it will allow for reducing bowel toxicity while maintaining toxicity to the more sensitive kidney.

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