Abstract. Antibodies directed against tumor necrosis factor (TNF)-α are clinically used for Crohn's disease, rheumatoid arthritis and psoriasis. TNF-α is also an important cytokine in radiotherapy because it mediates inflammatory responses in normal tissues. To study the influence of TNF-α inhibition on radiation toxicity, we used a well-established mouse model of kidney irradiation, where the portal also includes parts of the intestine. Mice were treated with single-fraction radiotherapy to the right kidney with doses of 8 or 10 Gy with or without the monoclonal TNF-α antibody infliximab injected i.v. in three doses. The kidney function was assessed by means of repeated 99mTc-dimercaptosuccinate scans during a maximum follow-up of 49 weeks. Treatment with infliximab significantly exacerbated radiation nephropathy at all time points, both in the 8 Gy and 10 Gy groups. The drug itself is not known to cause renal impairment. In the control group irradiated with 10 Gy, one mouse died from delayed radiation-induced intestinal toxicity. Skin reactions and general performance status were also similar across the groups. These data suggest that administration of infliximab concomitant to radiotherapy causes profound alterations in the development of kidney dysfunction. Importantly, other radiation-related toxicities were similar across all groups.

Several drugs, which are commonly prescribed for non-cancer-related medical conditions, might influence the development of radiation-induced side-effects. Examples include angiotensin-converting enzyme inhibitors (1), pentoxifylline (2) and statins (3). A new class of drugs that potentially interact with radiation effects are TNF-α antibodies, which are rapidly entering routine clinical practice after randomized trials demonstrated their value in Crohn's disease, ankylosing spondylitis, rheumatoid arthritis and psoriasis (4-9). TNF-α was previously shown to mediate inflammatory responses in irradiated normal tissues (10-12). Therefore one might speculate that inhibition of this cytokine could abrogate undesirable side-effects. To study the influence of acute phase TNF-α blockade on long-term radiation tolerance of radiosensitive organs, we used a well-established experimental mouse model of kidney irradiation (13, 14), where the portal also includes parts of the intestine. TNF-α is produced by macrophages, renal mesangial and tubular epithelial cells, and its neutralization was found to reduce glomerular inflammation and tubulointerstitial scarring in non-radiation-associated glomerulonephritis (15). The drug used for the present study was infliximab, a chimeric human-murine monoclonal IgG1-anti-TNF antibody, which after injection is distributed throughout the vascular compartment and binds to both soluble and transmembrane TNF-α to form a stable complex (16-18).

Materials and Methods

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Animals. Female adult C3H/N mice (12 weeks old and weighing approximately 22-25 g) 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) to a 1.5-cm wide single field that included the right kidney, but also the adjacent bowel (at a dose rate of approximately 6 Gy per minute and source skin distance of 10 cm). 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 already been described (13, 14). Radiation was administered in single fractions of 8 or 10 Gy, based on our previous experience (13). 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 anesthesia system to immobilize them in the desired position. The initial group size was 5-8 mice in the control group and 7-8 mice in the TNF-α antibody group.

Assessment of response. The kidney function was assessed prior to radiotherapy as well as 19 weeks thereafter and then every 6 weeks for a maximum of 49 weeks by means of 99mTc-dimercaptosuccinate (DMSA) scans in the Department of Nuclear Medicine. As already outlined, this static scintigraphic method acquires relative measurements of the kidney function without absolute quantitative information (13). However, this method allows for examination of the ipsilateral function after irradiation, which is expected to decline steadily from approximately 50% of the total function in healthy mice at baseline. The reproducibility of the scintigraphic measurements is in the order of 3% (13). Clinical follow-up included assessment of general performance status, body weight and irradiated skin twice weekly. Skin reactions were scored as "absent, mild, moderate and ulceration". Autopsy was performed in all mice that died during follow-up. 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 SPSS software (SPSS Inc., Chicago, IL, USA). A p-value of <0.05 was considered statistically significant.

Infliximab injection. In order to reduce the TNF-α effect during the acute phase of radiation-induced reactions, mice received 3 intravenous injections of infliximab (Essex Pharma, Munich, Germany) each with a dose of 8 mg/kg body weight into the tail vein (one day before radiation treatment, one day after and five days after irradiation). This dose was derived from other mouse models (16, 17, 19) and is expected to inhibit TNF-α for at least 9-10 days after the final injection because the serum half-life is approximately 10 days. The common human dosage is 5 mg/kg. Table I summarizes all groups that formed the basis of this experiment.

Results

Before irradiation, the right kidney contributed 49-58% to the total kidney function (median of all animals combined: 55%). Modest declines of the kidney function developed in control animals, while the infliximab groups showed pronounced nephropathy (Table I). Figures 1 and 2 show the continuous deterioration over time expressed as the relative function. In the 10 Gy group, one control mouse died after 20 weeks from intestinal obstruction. No other lethal toxicities were observed in any group. Acute and chronic skin reactions in the radiation field were absent or mild and indistinguishable between control mice and the infliximab groups (in this model, the skin dose is roughly 100% higher than the dose prescribed to the kidney, i.e. 16-20 Gy). General performance status and body weight were also similar across the groups.

Discussion

In the kidney, a variety of changes in different cell types occur after irradiation. They have been comprehensively discussed by several experts in the field (20-22) and will not be reviewed in greater detail here. Changes include increased vascular permeability, perfusion disturbance and fibrosis. Although our mouse model was developed to assess the time course of radiation nephropathy, in order 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 in our previous erythropoietin experiments, radiation doses of up to 10 Gy induced only limited kidney dysfunction (13). However, 10 Gy caused a single case of lethal bowel complications, which were not more frequent in the infliximab group. Importantly, there was also no influence of the drug on skin toxicity, general performance status or body weight, while kidney dysfunction was much more pronounced over the whole length of follow-up. The organ selectivity of the observed effect argues against simple explanations, such as the hypothesis that TNF-α might be necessary for the initiation of proper healing processes after irradiation while inhibition of the cytokine would result in impaired healing and increased damage.
Figure 1. Time course of the mean relative function of the irradiated right kidney (8 Gy) with or without infliximab. Baseline function defined as 100%. The differences are statistically significant.

Figure 2. Time course of the mean relative function of the irradiated right kidney (10 Gy) with or without infliximab. Baseline function defined as 100%. The differences are statistically significant.
The TNF-α antibody dose and frequency of administration was chosen from experiments in other animal models (16, 17, 19). Clinical administration schedules were less dose-dense (7). Yet, the process of drug development, preclinical testing and implementation via several large clinical trials provided no evidence for drug-induced renal toxicity. As detrimental effects in the present study were limited to the kidney, it appears likely that causes other than the experimental design need to be taken into account. These might include negative influences of infliximab on the vascular system, demonstrated recently in the setting of femoral artery occlusion in rabbits (23). Such effects might be mediated by reduced levels of vascular endothelial growth factor (VEGF) (24), a growth factor that exerts protective effects on irradiated spinal cord (25). Another important cytokine pathway, which unlike VEGF and TNF-α has been studied previously in irradiated kidney, is the transforming growth factor (TGF)-β pathway (20). In fibroblasts, TNF-α was found to suppress TGF-β-induced signalling (26, 27). Therefore, TNF-α blockade might allow for activation of pro-fibrotic cascades contributing to impaired kidney function. In the absence of immunohistochemical tissue examinations, the exact mechanism remains speculative. The safety of TNF-α inhibitors in radiotherapy patients, at least when used concomitantly to portals including the kidneys, needs to be assessed prospectively.

References


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