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Radiation nephropathy in a nonhuman primate model of partial-body irradiation with minimal bone marrow sparing. Part 1: acute and chronic kidney injury and the influence of Neupogen

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Abstract

Acute and chronic kidney injury may occur after accidental prompt radiation exposures. We have modeled their occurrence in a nonhuman primate model. Subjects who are exposed to more than 5 Gy prompt irradiation are apt to show blood cell cytopenias and be treated with granulocyte colony stimulating factors such as Neupogen® or Neulasta® to mitigate the hematologic injury of the acute radiation syndrome. Neupogen® or Neulasta® are now approved by the US FDA for this indication. This will significantly increase the number of survivors of acute radiation exposures, who will be at risk for delayed effects of radiation exposure, which includes acute and chronic kidney injury. The primary objectives of the present two companion manuscripts were to assess natural history of delayed radiation-induced renal injury in a nonhuman primate (NHP) model of acute high-dose, partial-body irradiation with 5% bone marrow (PBI/BM5) sparing, to include the clinical and histopathological evidence and the effect of Neupogen® administration on the morbidity and mortality.

Eighty-nine nonhuman primates underwent 10 or 11 Gy partial body irradiation with 5% bone marrow sparing, of which 36 were treated with Neupogen® within 1, 3, or 5 days post irradiation. All animals were followed up to 180 days after irradiation. Renal function and histology endpoints showed early acute and later chronic kidney injury. These endpoints were not affected by use of Neupogen®. We conclude that use of Neupogen® to mitigate against the hematopoietic acute radiation syndrome has no impact on acute or chronic kidney injury.

Keywords

Partial body irradiation; Non-human primates; Normal tissue radiation injury; Acute kidney injury; Chronic kidney injury

Introduction

The threat of accidental or belligerent radionuclear events mandates studies that clarify the early, delayed, and late effects of radiation exposure. Radiation nephropathy is well described in humans, and in canine, rodent, and non-human primate models (Cohen and Robbins 2003). These have emphasized the combined mechanistic roles of endothelial and parenchymal injury. Because the acutely irradiated subject will be treated for acute radiation syndrome (ARS), and because survivors of ARS may develop delayed injuries, the effects of early administration of medical countermeasures (MCM) on the development of the organ-specific injury characteristic of the ARS and delayed effects of acute radiation exposure (DEARE) require investigation.

A nonhuman primate (NHP) model of partial-body irradiation with approximately 5% hematopoietic bone marrow (BM5) sparing has been established. This models accidental or belligerent partial body irradiation (PBI) exposures as might occur to humans (MacVittie et al. 2012). This model of PBI is highly applicable to humans because of the use of NHP and because of the use of individualized patient management (MacVittie et al. 2012). After 10 or 11 Gy PBI/BM5, the NHP developed the ARS multi-organ injury within the first sixty days which is characterized by the acute gastrointestinal (GI) and hematopoietic (H) ARS. There is subsequent radiation-induced pneumonitis and fibrosis in addition to prolonged gastrointestinal (GI) injury. In addition, both acute and chronic kidney injury occur in this model (Cohen et al. 2017). Irradiated subjects are at high risk for injury to their hematopoietic system. Cytopenias occurring after irradiation are likely to be treated with the leucocyte growth factors (LGF such as filgrastim (Neupogen®) or pegfilgrastim (Neulasta®). The benefit of Neupogen® or Neulasta® to improve survival of NHP exposed to mid-lethal doses within the H-ARS has been shown. (Farese et al. 2014, Hankey et al. 2015). Because improvements in supportive care combined with administration of LGFs will enable survival of irradiated subjects beyond the ARS, there is increasing attention to the delayed effects of acute radiation exposures (DEARE), also known as late effects. It is essential to test whether the supportive measures plus administration of LGFs for the ARS have an impact on the concomitant other organ injuries within the ARS and the DEARE (MacVittie et al. 2015). Further, there is some controversy as to whether GCSF may influence acute kidney injury (Nishida and Hamaoka, 2006). In a previous report that used this model, we reported on 36 NHP that had undergone PBI/BM5 but had not received Neupogen (Cohen et al. 2017). Unexpected but clear-cut acute and chronic kidney injury were found in these NHP. These kidney injury endpoints were not expected when the experiments were initially designed. The US FDA has concerns regarding off target effects of medical countermeasures against radiation on other organ injuries. The present studies used the complete cohort of NHP that underwent 10 or 11 Gy PBI/BM5, to determine whether use of Neupogen® influenced the acute or chronic kidney injury after exposure using the PBI/BM5 protocol.

Materials and Methods

Animals

Male rhesus macaques (n=88), *Macaca mulatta*, [5.5 – 11.3 kg body weight (bw)] were exposed to PBI in doses of 10.0 or 11.0 Gy and sparing the tibiae, ankles and feet which have approximately five percent of the NHP bone marrow (PBI/BM5). Forty six NHP underwent 10 Gy, and 42 underwent 11 Gy PBI/BM5 exposures. Fifteen of the NHP that underwent 10 Gy PBI also had Neupogen® started at day 1 after PBI (n= 7) or at day 3 after PBI (n=8). Twenty one of the NHP that underwent 11 Gy PBI also had Neupogen® starting at day 1 (n=5), day 3 (n=8), or day 5 after PBI (n=8). The timing was chosen to test the mitigating benefit of Neupogen on the acute H-ARS, and has been reported previously (MacVittie et al, 2015). An additional six were non-irradiated controls. All irradiated NHP were observed for up to 180 days post-irradiation, or until they were euthanized according to published criteria (MacVittie et al. 2012, MacVittie et al, 2015).

All NHP were in good health at start of study, were free of simian immunodeficiency virus, simian T cell leukemia virus type 1, malaria, herpes B virus, and tuberculosis. All animal procedures were performed according to an approved Institutional Animal Care and Use Committee (IACUC) protocol.

Housing, Care, Food and Water:

Animal housing and care was performed in accordance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*. Irradiated animals were single-housed in stainless steel cages at the University of Maryland animal facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Anesthesia: Ketamine (Ketaset, Fort Dodge, IA) ($10 \pm 5 \text{ mg kg}^{-1}$), either alone or in combination with xylazine (AnaSed, Fort Dodge, IA) ($1 \pm 0.5 \text{ mg kg}^{-1}$) was given intramuscularly (IM) to sedate animals before procedures. Yohimbine (Yobine, Shenandoah, IA) [$(0.2 \pm 0.1 \text{ mg kg}^{-1}$, IM or intravenously (IV))] was given to reverse xylazine sedation, if required.

Use of Neupogen®

Neupogen® (Amgen, Inc. Thousand Oaks, CA) was dosed according to the body weight at 10 mcg kg^{-1} and injected subcutaneously starting on day 1, 3, or 5 after PBI. Neupogen® was stopped when the absolute neutrophil count was $\geq 1000/\text{microliter}$ for three consecutive days. The median time of Neupogen® use was 11 days (MacVittie et al, 2015).

Radiation Exposure and Dosimetry

PBI was in doses of 10 or 11 Gy with 6 megavolt (MV) linear accelerator derived photons. Calibration and dosimetry were done as previously reported (MacVittie et al. 2012). PBI was delivered to midline tissue at an approximate dose rate of 0.80 Gy min^{-1} .

Individualized Medical Management

Cage-side observations, clinical observations, analgesics, anti-emetics, anti-ulcerative, anti-diarrheals, antibiotics, anti-inflammatories, anti-pyretics, diuretics, nutritional support, blood transfusions, and enteral or parenteral fluid support were provided as previously reported (MacVittie et al. 2012).

Euthanasia

Animals were euthanized based on criteria previously reported (MacVittie et al. 2015), and all surviving animals were euthanized at 180 ± 10 d post-irradiation. This was in compliance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals and our IACUC.

Blood chemistry

Blood was taken by saphenous vein venipuncture under ketamine anesthesia just before euthanasia. BUN and serum creatinine were determined using commercial kits on an Alfa Wassermann Ace Clinical Chemistry Analyzer (4 Henderson Dr., West Cladwell, NJ), the latter using the Jaffé reaction. Plasma creatinine was confirmed by liquid chromatography - tandem mass spectrometry (LC-MS/MS) in a subset of 53 animals using the Biocrates AbsoluteIDQ p180 kit (Biocrates, Life Science AG, Innsbruck, Austria) on a Shimadzu Prominence UFLC XR high-performance liquid chromatograph (HPLC) (Shimadzu, Columbia, MD) coupled to an AB Sciex QTRAP® 5500 hybrid tandem quadrupole/linear ion trap mass spectrometer (AB Sciex, Framingham, MA). Normal values were confirmed using blood samples from age-matched non-irradiated NHP.

Renal histology

Organs procured at necropsy were processed for histology. All samples were immersed in 10% neutral-buffered formalin for at least 48 h, embedded in paraffin, cut in 5 micron sections, and stained with Masson Trichrome. Slides were scanned using a Hamamatsu Nanozoomer HT slide scanner (Hamamatsu Corporation, Bridgewater, NJ) and viewed using the Aperio ImageScope software (Leica Biosystems, version 12.3.0.5056, Buffalo Grove, IL). Scoring was done in a masked fashion, without knowledge of the use of Neupogen®.

Acute kidney injury (AKI): AKI was scored for specimens obtained during the first 50 days after PBI/BM5. This is kidney injury occurring early after irradiation, which is consistent with the timing of acute kidney injuries of any cause, such as volume depletion, sepsis, or exposure to nephrotoxins. The acute injury score was for tubular injury (none, scattered, or diffuse) glomerular thromboses (none, scattered, or diffuse) and medullary congestion (none, scattered, or diffuse), each grade being 0, 1, or 2 for a maximum score of 6. There were 30 NHP in this day 0 to 50 group, of which eleven had no scanned tissue sections. The six additional NHP that were not irradiated also had scoring for acute kidney injury.

Chronic kidney injury (CKI): CKI was scored for specimens obtained from 50 to 180 days after PBI/BM5. This is kidney injury occurring in a delayed fashion after irradiation,

which is consistent with the known time course of radiation nephropathy in other models (Cohen and Robbins 2003). This histological scoring was for cysts (none, micro-, or macroscopic), interstitial fibrosis (none, scattered, or diffuse) casts (none, scattered, or diffuse) glomerular thrombosis (none, few, or most glomeruli of 20 examined), glomerulosclerosis (none, 1–2, 3–4, >4 of 20 examined), each grade being 0, 1, or 2, and also for mesangiolysis (none, variable, most, or all glomeruli of 20 examined), each grade being 0, 1, 2, or 3, for a maximum score of 14. Fibrosis was a prominent feature and was thus scored for glomeruli and interstitium. There were 58 NHP in this day 51 to 180 group, of which nine had no scanned tissue sections. The six additional NHP that were not irradiated also had scoring for chronic kidney injury.

Experimental endpoints

The primary endpoints were renal function, as azotemia, and the acute and the chronic histological injury score.

Statistical Methods

Continuous data are shown as geometric means. These are compared between groups using non-parametric statistics. Correlations were tested by Spearman tests. P values of = or < 0.05 were deemed significant (GraphPadPrism, San Diego, CA).

Results

Six irradiated animals did not have BUN values analyzed at time of euthanasia, whereas all irradiated animals had creatinine values analysis performed. The correlation of all creatinine values to BUN values was very good (Spearman $r=0.8$). A good correlation of the creatinine to renal tissue injury was previously shown in this model (Cohen et al. 2017). Creatinine rather than BUN was therefore used as the renal function marker for the present report. There was a good correlation of the creatinine values by the Jaffé reaction and the subset done by mass spectrometry ($r=0.8$). The median creatinine of the non-irradiated NHP was 0.6 mg/dl. This is near the average serum creatinine of 0.8 mg/dl for normal male NHP published elsewhere (Chen et al. 2009).

Within the first 20 days after PBI, all the creatinine values at euthanasia were higher than the median creatinine of the non-irradiated NHP (Fig. 1). This is consistent with acute kidney injury (AKI). Thereafter, values declined towards the normal range by approximately day 50 after irradiation. Creatinine values were again above the normal range starting at approximately 100 days after irradiation. The AKI phase was therefore determined to be the first 50 days after irradiation and the CKI phase was from day 51 through the end of the studies, at day 180 after irradiation (Cohen et al, 2017).

Renal function during the acute kidney injury phase

During this phase, the creatinines of the NHP that underwent 10 Gy and had Neupogen® administration starting at 1 or 3 days after PBI/BM5 were higher in the former group compared to the latter, averaging 1.7 and 1.1 mg/dl, respectively. Statistical comparison was not done because there are only two NHP in each of those groups. The creatinines of the 11

Gy NHP that had Neupogen® administration starting on day 1, 3, or 5 did not differ during this phase (ANOVA, $p = 0.6$). The values for NHP receiving Neupogen® were therefore combined for each dose group. The creatinines of the NHP that underwent 10 Gy did not differ according to whether they also were treated with Neupogen® ($p = 0.2$, Mann-Whitney). The creatinines in the NHP that underwent 11 Gy did not differ according to whether they also were treated with Neupogen® ($p = 0.4$, Mann-Whitney). The creatinines of the 11 Gy NHP were lower than the creatinines of the 10 Gy NHP (geometric mean of 0.7, 95% confidence interval (c.i.), 0.6 to 1.0 and 0.8, 95% c.i. 0.7 to 1.0, respectively) but this was not significant ($p = 0.3$). There are no visual trends that show an effect of either irradiation dose or use of Neupogen® on renal function in this acute phase (Fig. 2).

Renal function during the chronic kidney injury phase

Starting at 100 days after PBI, all the creatinine values at euthanasia except one were above the median creatinine of the non-irradiated NHP (Fig. 1). This shows chronic kidney injury, which is a known late effect of irradiation (Cohen and Robbins 2003). There is a general increase of creatinine values from day 100 onward and the median creatinine value at the end of the study 180 day time point was 1.2 mg/dl, i.e. twice the value of the non-irradiated NHP and consistent with a persistent and two-fold or more reduction in renal function.

During this chronic phase, the creatinines of the NHP that underwent 10 Gy and had Neupogen® administration starting at 1 or 3 days after PBI/BM5 did not differ ($p = 1$, Mann-Whitney). The creatinines of the 11 Gy NHP that had Neupogen® administration starting on day 1, 3, or 5 did not differ during this phase ($p = 0.9$, ANOVA). The values for NHP receiving Neupogen® were therefore combined for each dose group. The creatinines in the NHP that underwent 10 Gy did not differ according to whether they also were treated with Neupogen® ($p = 0.6$, Mann-Whitney). The creatinines in the NHP that underwent 11 Gy did not differ according to whether they also were treated with Neupogen® ($p = 0.5$, Mann-Whitney). The average of the creatinines of the 11 Gy NHP were similar to the creatinines of the 10 Gy NHP (geometric mean of 1.1, 95% c.i. 0.9 to 1.2, and 1.1, 95% c.i. 1.0 to 1.4., respectively). ($p = 0.9$). There are no visual trends that show an effect of either irradiation dose or use of Neupogen® on chronic kidney injury (Fig. 3).

Histological injury during the acute kidney injury phase

Within the first 20 days after PBI, 74% of the AKI injury scores at euthanasia were higher than the median AKI score of the non-irradiated NHP (Fig. 4). This is consistent with AKI (Cohen et al, 2017). This injury is evident as renal epithelial tubular injury, with vaculation of the cytoplasm and loss of definition of the brush border (Fig. 5). The normal renal histology of a non-irradiated NHP is shown for comparison (Fig. 6).

There were three NHP that underwent 10 Gy PBI/BM5 and were given Neupogen®, one starting at day 3 and two starting at day 1 after PBI. These had AKI injury scores of 2, and 4 and 3, respectively, and were not compared statistically. The AKI scores of the NHP that underwent 11 Gy and had Neupogen® starting on day 1, 3, or 5 did not differ ($p = 1$, ANOVA). The values for NHP that had Neupogen® were therefore combined for each dose group. The AKI scores in the NHP that underwent 10 Gy did not differ according to whether

they also were treated with Neupogen® ($p = 0.8$, Mann-Whitney). The AKI scores in the NHP that underwent 11 Gy did not differ according to whether they also were treated with Neupogen® ($p = 0.6$, Mann-Whitney). The AKI scores of the NHP exposed to 11 Gy were lower than the AKI scores of the NHP exposed to 10 Gy (geometric mean of 3.0, 95% c.i. 2.1 to 4.3, and 1.8, 95% c.i. 1.3 to 2.5, respectively) but this difference was not significant. ($p = 0.06$). There are no visual trends that show an effect of either irradiation dose or use of Neupogen® on acute kidney injury (Fig. 4).

Histological injury during the chronic kidney injury phase

Starting at 100 days after PBI, all of the CKI injury scores at euthanasia were higher than the median CKI score of the non-irradiated NHP (Fig. 7). Chronic kidney injury was evident as prominent glomerular and interstitial scarring (Fig. 8). Narrowing of the junction between the glomerular urinary space and its draining tubule was a definite feature (Fig. 9, which is a known feature of other models of radiation nephropathy (Cohen et al. 1997, Cohen et al. 2000). The normal renal histology of a non-irradiated NHP is shown for comparison (Fig. 6)

The chronic kidney injury scores of the NHP that underwent 10 Gy and had Neupogen® administration starting at 1 or 3 days after PBI/BM5 did not differ ($p = 0.5$, Mann-Whitney). The chronic kidney injury scores of the NHP that underwent 11 Gy and had Neupogen® starting at 1, 3 or 5 days after PBI/BM5 did not differ ($p = 0.3$, ANOVA). The values for NHP receiving Neupogen® were therefore combined. The CKI scores in the NHP that underwent 10 Gy did not differ according to whether they also were treated with GCSF ($p = 0.6$, Mann-Whitney). The CKI scores in the NHP that underwent 11 Gy did not differ according to whether they were treated with Neupogen® ($p = 0.9$, Mann-Whitney). The injury scores of the NHP exposed to 11 Gy were lower than the AKI scores of the NHP exposed to 10 Gy during this chronic phase (geometric mean of 3.8, 95% c.i. 3.1 to 4.9, and 5.0, 95% c.i. 4.5 to 5.7, respectively) but this was not significant. ($p = 0.2$). There are no visual trends that show an effect of either irradiation dose or use of Neupogen® on chronic kidney injury (Fig. 7).

Correlations of histological injury scores and azotemia

There was no significant correlation of the AKI histological score with azotemia, expressed as creatinine ($r = 0.3$, $p = 0.2$). There was a significant direct correlation of the CKI histological score with azotemia, expressed as creatinine ($r = 0.4$, $p = 0.01$).

Discussion

These studies show that use of Neupogen® during the acute radiation syndrome has no effect on either acute or chronic kidney injuries that develop after 10 or 11 Gy PBI/BM5.

Neupogen® will stimulate hematopoietic cell lineages and in retrospect one might expect that these could exert a pro-inflammatory effect that could worsen acute or chronic kidney injury. But radiation nephropathy does not have a major cellular inflammatory component (Mostofi, Pani & Ericsson 1964, Cohen 2000, van Kleef et al. 2000). Thus the enhanced recovery of neutrophil blood counts caused by use of Neupogen® would thus not be expected to worsen the AKI or the CKI.

The lack of correlation of the AKI histological scores to azotemia is not surprising. This lack of correlation was reported years ago (Finckh et al 1962). In addition, the low “n” of the AKI cases in the present report could explain a lack of structure-to-function correlation even if one existed. We did however find a significant and direct correlation of the CKI histological score to the degree of azotemia. This is consistent with recent reports that correlate the extent of chronic injury and also of renal fibrosis found on renal biopsies with prognosis for the development of complete renal failure (Howie et al. 2001).

The AKI phase appears to show injury and azotemia during the first few weeks after irradiation. Tubular injury was a major feature of AKI, rather than glomerular, which was confirmed as reported by Parker et al (Parker, Cohen, 2018, herein). This AKI is during a time when the acute gastrointestinal radiation syndrome is severe and there is the resulting volume depletion (dehydration) (Cohen et al. 2017). That volume depletion will cause pre-renal azotemia and also acute tubular necrosis. The former resolves with the provision of sufficient parenteral fluids so that the latter resolves with time, provided that the affected subject does not die from a complication of AKI such as hyperkalemia, pulmonary edema, or bleeding. In the AKI of this model, the creatinine levels decline progressively after their early peak, consistent with improvement in renal function. The AKI histological injury scores may also show an initial peak then a decline, if one excludes the one outlier at 41 days.

The CKI phase is progressive, as shown by the increasing azotemia and CKI histological injury scores during the course of the study. Its time course is consistent with radiation nephropathy seen in an 11 Gy single fraction model in rats (Moulder et al. 2011) and also consistent with the BMT-related radiation nephropathy congener in humans (Antignac et al. 1989). Had these studies continued for longer than 180 days, we expect that there would be morbidity and mortality caused by CKI itself.

In retrospect, it is difficult to envisage how the use of Neupogen® during the ARS phase could have a later influence on chronic kidney injury that is evident at 100 days and increasingly severe thereafter. A worsening of the later CKI by Neupogen® might occur because of an early stimulation of pro-fibrotic factors such as transforming growth factor beta 1 or connective tissue growth factor, both of which have been implicated in the fibrosis of radiation nephropathy (Cohen et al. 2000, Zhang et al. 2015). But there is no evidence that Neupogen® acts to upregulate either of these cytokines.

Mast cells are often found in areas of tissue fibrosis and may have a mechanistic role in progressive kidney diseases (Roberts and Brenchley 2000). Tryptase-stained kidneys from the present studies show mast cells in kidneys showing CKI, especially at the 180 day end-of-study time point (Parker, Cohen, 2018, herein). It is possible that Neupogen® could stimulate mast cell production by the hematopoietic marrow, but this effect would have to last well beyond the last dose of Neupogen®, which was typically only a few weeks after PBI/BM5.

There may be additional mechanistic links between the ARS and the renal DEARE. Currently, much attention is being placed on whether AKI evolves into CKI or at least

potentiates its development. (Heung et al. 2016). We showed that the AKI in this model is likely to be the consequence of severe volume depletion during the first several weeks after PMI/BM5, which in turn is secondary to gastrointestinal injury that causes nausea, vomiting, and diarrhea (Cohen et al. 2017). Our individualized and trigger-based treatment of this volume depletion is likely to have rescued many of the NHP from its acute effects, thus enabling their longer term survival in which the later DEARE and CKI could become manifest. But we found no correlation between the severity of the early volume depletion and the severity of the later CKI.

Long-term use of granulocyte colony stimulating factors in humans with chronic neutropenia has been associated with the rare occurrence of proliferative glomerulonephritis (Dale et al. 2003). There was no histological appearance of glomerulonephritis in any of the renal scanned sections of these studies, which is confirmed by Parker et al (Parker, Cohen, 2018, herein).

Conclusions

Radiation exposures are apt to cause more than one organ injury. Thus, a predominantly upper body exposure could injure the upper GI tract and lungs, whereas a lower body exposure would damage GI tract and kidneys. Both would have cutaneous and hematopoietic effects. It is unlikely that a single treatment will be effective against any organ injured by radiation. Different medical countermeasures, used at different times, are needed, much as they have been for human radiation accident victims. These range from early use of intravenous fluids and antibiotics to the later use of corticosteroids. The FDA approved Neupogen® and its congeners are very likely to be used in irradiated human subjects who are leukopenic within the first few days of exposure, with the intent of achieving their long term survival. Because single fraction exposures of more than 6 Gy may cause renal injury, it is therefore essential to test the potential “off-target” effects of the medical countermeasures to contribute to both acute and chronic kidney injury. Long-term use of Neupogen® appears safe in human use (Dale et al. 2017). Neupogen® used for the acute radiation syndrome also appears to be safe, and to have neither adverse nor beneficial early or late renal effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 2.

Values of the creatinine at time of euthanasia during the first 50 days after irradiation. The values are shown for NHP that underwent 10 Gy or 11 Gy PBI/BM5 with no use of Neupogen® (0), Neupogen® starting at day 1 (1), day 3 (3), or day 5(5). The dotted grey line shows the value of creatinine in non-irradiated NHP. The creatinine is elevated within the first ten days, then appears to fall with time, which is consistent with early acute kidney injury that resolves over the ensuing weeks. The response to 10 Gy and to 11 Gy is similar, probably because their effect is indirect, via the extracellular volume depletion that results from acute gastrointestinal injury. There appears to be no influence of the use, or not, of Neupogen® on this acute kidney injury.

Figure 3.

Values of the creatinine at time of euthanasia starting at 100 days after irradiation and until end-of-study at 180 days. The values are shown for NHP that underwent 10 Gy or 11 Gy PBI/BM5 with no use of Neupogen® (0), Neupogen® starting at day 1 (1), day 3 (3), or day 5(5). The dotted grey line shows the value of creatinine in non-irradiated NHP. There is a progressive elevation in creatinine, consistent with chronic kidney injury. The response to 10 Gy and to 11 Gy is similar, although earlier loss of 11 Gy NHP may obscure the expected dose-response relationship. There appears to be no influence of the use, or not, of Neupogen® on this chronic kidney injury.

Figure 4.

The acute kidney injury histological score at time of euthanasia during the first 50 days after irradiation. The values are shown for NHP that underwent 10 Gy or 11 Gy PBI/BM5 with no use of Neupogen® (0), Neupogen® starting at day 1 (1), day 3 (3), or day 5(5). The dotted grey line shows the value of injury score in non-irradiated NHP. The injury score is elevated within the first thirty days, then appears to fall with time, except for one outlier, which is consistent with early acute kidney injury that resolves over the ensuing weeks. The response to 10 Gy and to 11 Gy is similar, probably because their effect is indirect, via the extracellular volume depletion that results from acute gastrointestinal injury. There appears to be no significant influence of the use, or not, of Neupogen® on this acute kidney injury.

Figure 5. Acute kidney injury histology. Masson-trichrome stained kidney section, 500x magnification. There is renal tubular epithelial injury, with loss of definition of the brush border (a), loss of definition of epithelial cell nuclei (b), some loss of tubular cell volumes, and intracellular vacuole formation (c). There is a modest increase in glomerular matrix (d). There is scant intertubular blue staining fibrosis. There is no inflammation.

Figure 6.

Normal renal histology. Masson-trichrome stained kidney section, 500x magnification. The glomeruli (g) have normal cellularity and patent capillary loops. The renal tubular epithelium (rte) is intact, with normal cell nuclei and brush borders. The tubules are back-to-back, with no intervening fibrosis.

Figure 7.

The chronic kidney injury histological score at time of euthanasia starting at 100 days after irradiation and until end-of-study at 180 days. The values are shown for NHP that underwent 10 Gy or 11 Gy PBI/BM5 with no use of Neupogen® (0), Neupogen® starting at day 1 (1), day 3 (3), or day 5(5). The dotted grey line shows the value of injury score in non-irradiated NHP. There is a progressive increase in chronic kidney injury in this study, consistent with typical radiation nephropathy in other animal models. The response to 10 Gy and to 11 Gy is similar, although earlier loss of 11 Gy NHP may obscure the expected dose-response relationship. There appears to be no significant influence of the use, or not, of Neupogen® on this chronic kidney injury.

Figure 8. Chronic kidney injury histology. Masson-trichrome stained kidney section, 500x magnification. There is abundant blue-staining interstitial collagenous fibrosis (f) that separates the renal tubules. There are only scant interstitial mononuclear inflammatory cells. The glomerular tufts show increased purple staining matrix (m), with loss of cell numbers and loss of capillary lumens. There are red-staining glomerular thrombi (t).

Figure 9. Chronic kidney histology. Masson-trichrome stained kidney section, 500x magnification. There is abundant blue-staining interstitial collagenous fibrosis that separates the renal tubules. This appears accentuated at the start of the proximal tubule, forming a stenotic glomerulo-tubular neck (arrow). There are only scant interstitial mononuclear inflammatory cells.