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ORIGINAL ARTICLE

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Accelerated recovery from irradiation injury by angiotensin peptides

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Abstract *Purpose:* Angiotensin peptides have been shown to affect the proliferation and chemotaxis of multiple cell types. More recent studies in this laboratory have shown that angiotensin II (AII) can increase colony formation and proliferation by hematopoietic progenitors and mesenchymal cells in vitro. As white blood cell (WBC) recovery after bone marrow injury requires progenitor proliferation, the effect of AII and angiotensin (1–7) [A(1–7)], a non-hypertensive fragment of AII, on recovery from total body irradiation was evaluated in C57Bl/6 mice. *Materials and methods:* The effect of angiotensin peptides on hematopoietic recovery and the number of progenitors in the bone marrow of irradiated C57Bl/6 mice was evaluated. *Results:* Treatment of animals with angiotensin peptides accelerated hematopoietic recovery and increased the number of hematopoietic progenitors in bone marrow and in the blood. The increase in WBC concentration continued for a longer time after cessation of AII therapy than after treatment with filgrastim. Specifically, the number of WBCs continued to increase 21 days after irradiation with 7 days of angiotensin peptide administration. In contrast, the number of WBCs increased through day 13 with 7 days of filgrastim administration. On day 35 after irradiation (28 days after the last treatment), AII was shown to have increased the number of CFU-GM in the bone marrow of irradiated mice, whereas filgrastim administration had not. Angiotensin peptides also reduced the drop in platelet concentration after irradiation and increased the number of megakaryocyte precursors and megakaryocytes in the bone marrow. Receptor blocking studies indicated that losartan, an antagonist of the angiotensin type 1 receptor, blocked recovery of WBC levels in response to treatment with AII. In contrast, the increase in WBC levels in response to treatment with A(1–7), a ligand for other angiotensin receptors, was not affected by losartan. *Conclusions:* These findings suggest that these peptides utilize distinct receptors in the stimulation of hematopoietic recovery. In summary, systemic administration of angiotensin peptides led to an acceleration in hematopoietic recovery after irradiation. These peptides act to stimulate the formation of bone marrow progenitors, thereby facilitating recovery after myelosuppressive irradiation.

Keywords Irradiation · Myelosuppression · Angiotensin · Marrow hematopoiesis

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Introduction

Angiotensin II (AII) is a peptide that has been traditionally recognized as a regulator of blood pressure, salt, and water homeostasis. However, recent studies have shown that AII and angiotensin (1–7) [A(1–7)], a non-hypertensive fragment of AII, are potent regulators of tissue regeneration [35, 36, 39]. Studies over the past decade have shown that AII and A(1–7) also have regulatory effects on cellular proliferation and growth factor release, which may contribute to accelerated tissue regeneration. AII has been shown to act as a mitogen for smooth muscle cells, fibroblasts and endothelial cells [2, 7, 13, 14, 17, 33, 34, 40, 41]. AII also increases the protein content and size of vascular smooth muscle cells and cardiomyocytes [3, 4, 18]. A(1–7) is a peptide that shares some of the properties of AII, but can also oppose some of the actions of AII. For example, A(1–7) has been shown to inhibit the proliferation of smooth muscle cells, but to increase fibroblast and keratinocyte proliferation [14, 15, 17, 39].

A(1–7) is a member of the renin angiotensin system (RAS) that includes the first seven amino acids of AII and, importantly, does not induce hypertension. While AII is derived from AI by angiotensin-converting enzyme, A(1–7) is the product of AI cleavage by neutral endopeptidases [24]. Recent studies have shown this fragment to be active in several systems including renal function and wound repair [24, 39].

In more recent studies, AII has been shown to increase the release of growth factors of various types, including platelet-derived growth factor (PDGF), heparin-binding epidermal growth factor, basic fibroblast growth factor, endothelin-1 and transforming growth factor-β (TGF-β), increases growth factor gene expression, and increases expression of growth-related proto-oncogenes, c-myc, c-fos, c-jun and erb-1, in cultures of smooth muscle cells, endothelial cells and cardiac fibroblasts [8, 20, 21, 22, 26, 27, 31, 44, 47]. AII has also been shown to increase growth factor receptors. Some of the effects of AII on cell function are mediated through alterations in growth factor production and may be additive with the effects of growth factors per se. The release of TGF-β has been shown to mediate some of the effects of AII on vascular smooth muscle cells. The addition of neutralizing antibodies to TGF-β to cultures exposed to AII blocks the proliferative effects of AII.

During studies assessing the effect of AII on tissue healing after partial thickness burning, infiltration of mesenchymal stem cells into the burn site has been observed during histological evaluation of thermal injury repair. Further, these histological studies have shown that AII and A(1–7) may increase the proliferation of epidermal stem cells in the base of the hair follicle [36, 39]. Recent studies in this laboratory have shown that AII stimulates the proliferation of human and murine hematopoietic progenitors in vitro [37]. Recovery of bone marrow and circulating white blood cells (WBC) after irradiation is a form of tissue regeneration that requires the proliferation of progenitor cells and may be augmented by growth factors. Therefore, the ability of angiotensin peptides to act as hematopoietic factors after irradiation was evaluated and compared with that of filgrastim, a well-known stimulator of hematopoietic recovery with the ability to mobilize hematopoietic precursors, in a murine model. The effect of systemic administration of AII and A(1–7) on the level of myeloid, erythroid and megakaryocytic progenitors cells and megakaryocytes in the bone marrow of irradiated mice was also evaluated.

AII was evaluated because of its known effects on the proliferation of hematopoietic and erythroid progenitors [30, 37]. However, the clinical utility of AII would be limited by the effects of this peptide on blood pressure. As A(1–7) also stimulates the proliferation of presumably epidermal stem cells in the base of the hair follicle after partial thickness injury in guinea pigs and does not induce hypertension, the effect of this peptide on hematopoietic recovery was also evaluated.

Materials and methods

Materials

AII and A(1–7) were purchased from Bachem (Torrance, Calif.) and manufactured under "good manufacturing practice" conditions. Filgrastim (Neupogen) was purchased in a pharmaceutical preparation (Amgen, Thousand Oaks, Calif.). All chemicals to assess the number of circulating formed elements were of reagent or tissue culture grade and were purchased from Sigma Chemical Company (St. Louis, Mo.). Losartan was a gift from DuPont Merck (Wilmington, Del.). The materials for in vitro assessment of progenitors were purchased from Stem Cell Technologies (Vancouver, BC).

Irradiation model

Female C57Bl/6 mice (Jackson Laboratories, Bar Harbor, Me.) were irradiated with 600 cGy total body irradiation (TBI) with a cesium irradiator at the Norris Cancer Hospital (Los Angeles, Calif.). In an initial study, subcutaneous injection with either AII (10 or 100 μg/kg per day) or saline (placebo) was initiated 2 days before, on the day of (day 0) or 2 days after irradiation and continued until the animals were killed. In this initial study, the animals that died, died late after day 9 after irradiation indicating that the mortality was the result of bone marrow failure and infection rather than anemia and platelet loss. Mortality did not occur in subsequent studies at this same dose of irradiation, suggesting that this dose of irradiation is on the borderline of a lethal dose.

In a subsequent study, AII or filgrastim was administered for shorter periods (0–2 or 0–7 days) and effects on WBC recovery compared. In all further studies, administration of both peptides was initiated on the day of irradiation or 2 days after and continued until the animals were killed. In other studies losartan (50 μg/kg per day) was administered with the peptides daily, starting 2 days after irradiation. At various times after irradiation, the mice were anesthetized with methoxyflurane and bled via the retro-orbital sinus. The collected blood was mixed with red blood cells (RBC) lysing solution (0.83% NH_4Cl, 10 m*M* EDTA, 0.5% $NaHCO_3$) for 10 min at 4°C. The cells were centrifuged at 2000 rpm for 20 min and the pellet was resuspended in phosphate-buffered saline (PBS). The number of WBCs was determined on a hematocytometer under light microscopy. The number of platelets was determined on a hematocytometer under phase-contrast microscopy. Aliquots of cells were also prepared for differential analysis by cytocentrifugation onto a glass slide followed by staining with Giemsa. The cell types present in the peripheral blood were determined by assessment of cell morphology under light microscopy.

Evaluation of myeloid and erythroid progenitors in bone marrow

On the day the animals were killed, the number of myeloid and erythroid progenitors in the bone marrow was evaluated. The animals were killed, the femurs removed and flushed with 2 ml PBS (pH 7.2) containing 1% bovine serum albumin (BSA). After allowing debris to settle and centrifugation to pellet the cells, the supernatant was removed and the cells resuspended in 5 ml RPMI-1640 containing 10% fetal calf serum (FCS) [11, 12, 28].

After counting the viable cells by trypan blue exclusion via a hematocytometer, an aliquot of the cells was diluted to 1×10^5 cells/ml. After mixing 100 μl of this cell suspension with 900 μl methyl cellulose medium comprising Iscove's modified Dulbecco's medium (IMDM) containing 0.9% methyl cellulose, 15% FCS, 1% BSA, 10 μg/ml insulin, 200 μg/ml glutamine, 10 ng/ml recombinant murine interleukin-3 (IL-3), 10 ng/ml recombinant human IL-6, 50 ng/ml recombinant mouse stem cell factor, and 3 U/ml recombinant murine erythropoietin (Stem Cell Technology, Vancouver, BC), 1 ml of the mixture was placed in the wells of 24-well plates. This medium supported the growth of granulocyte macrophage colony forming units (GM-CFU), granulocyte erythroid

megakaryocyte macrophage colony forming units (GEMM-CFU) and burst forming units erythroid (BFU-E). The cultures were incubated for 14 days at 37°C in a humidified atmosphere of 5% CO_2 in air. After 14 days, the number of GM-CFU, GEMM-CFU and BFU-E per well was microscopically evaluated.

Evaluation of CFU-megakaryocyte in the bone marrow

Cells were isolated from the bone marrow as described above. After enumeration of viable nucleated cells, an aliquot of the cells was resuspended to a concentration of 1×10^6 cells/ml, and 100 µl of this suspension was mixed with 2 ml of medium containing 1.1 mg/ml collagen, 1% BSA, 10 µg/ml bovine pancreatic insulin, 200 µg/ml human transferrin, 2 m*M* L-glutamine, 10 µg/ml 2-mercaptoethanol, 50 ng/ml recombinant human thrombopoietin, 20 ng/ml recombinant human IL-6, 50 ng/ml recombinant human IL-11, 100 ng/ml recombinant murine IL-3 in IMDM (MegaCult-C; Stem Cell Technologies). The cells and medium were mixed and dispensed onto tissue culture slides, spread evenly and allowed to gel. The culture slides were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 8 days.

At the end of the culture time, the formation of megakaryocytes from megakaryocyte precursors (CFU-Meg) was determined by staining for the expression of acetyl cholinesterase [23]. The culture slides were opened and the collagen gel was fixed in an acetone solution and air-dried. The substrate solution (0.5 mg/ml acetylthiocholiniodide in 0.075 *M* sodium phosphate buffer, 0.01 *M* sodium citrate, 3 m*M* copper sulfate and 0.5 m*M* potassium ferricyanide solution) was added to the fixed slides and allowed to incubate in a humid chamber for 3.5 h. The slides were then fixed in a solution of 95% ethanol for 10 min, rinsed and air-dried. The slides were then counterstained with hematoxylin solution for 30 s, rinsed and dried.

Statistics

The statistical significance of these results was evaluated by the Chi-squared (survival) test, Student's test and Duncan's multiple range test. *P*-values ≤ 0.05 were considered significant.

Results

Hematopoietic recovery by angiotensin peptide after irradiation

Initial studies with AII showed that initiation of exposure to this peptide on either day 0 or day 2 after irradiation protected against mortality resulting from bone marrow failure (Table 1). This was most likely due to more rapid restoration of circulating WBC (Fig. 1a). The types of WBC restored to the periphery by systemic daily administration of AII were examined microscopically. In the animals exposed to AII starting on the day of irradiation, the cells that recovered included neutrophils (Fig. 1b), monocytes (Fig. 1c), lymphocytes (Fig. 1d) and, unexpectedly, megakaryocytes (data not shown). A(1–7) also increased the numbers of WBC when administration was initiated on the day of irradiation (Fig. 2).

Comparison with filgrastim

These irradiation studies were repeated but with AII (1, 10 or 100 µg/kg per day) administered for shorter time periods (0–2 or 0–7 days after irradiation) and compared with subcutaneous injection of filgrastim at the same doses. Administration of both agents (AII or filgrastim) increased circulating WBC levels after injection, but the benefit from AII administration increased with time and was more pronounced at lower doses (Fig. 3).

Differential counts were conducted on the peripheral blood cells. As with the number of WBC, the number of peripheral blood neutrophils, monocytes and lymphocytes (Table 2) in the AII-treated animals did not decrease to the same level as in the control animals. As with WBC, the nadirs in neutrophil and lymphocyte concentrations occurred on day 7 after AII and filgrastim treatment. As with WBC concentration, the effect of AII on neutrophil recovery continued through day 21. In contrast, no effect of filgrastim was observed after day 13.

Effect of losartan on the hematopoietic recovery induced by angiotensin peptides

These studies were conducted to determine whether AII and A(1–7) act to promote hematopoiesis through the AT_1 receptor, the receptor commonly associated with cellular proliferation. As before, angiotensin peptides accelerated hematopoietic recovery after irradiation (Fig. 4). Coadministration of losartan with the peptides blocked the recovery induced by AII (Fig. 4a), but did not affect the recovery induced by A(1–7) (Fig. 4b).

Effect of angiotensin peptides on myeloid and erythroid progenitors in the bone marrow

In initial studies, the effect of AII on the number of myeloid progenitors in the bone marrow was compared with that of filgrastim. In this study, the peptide was injected on day 0 through day 7 after irradiation and the animals were killed 28 days later (day 35 after irradiation). As shown in Table 3, administration of AII increased the number of GM-CFU in the bone marrow, whereas filgrastim did not. In a further study, both AII

Table 1. Effect of administration of AII on survival of irradiated recipients (presented as percent survival). The data presented were from five animals per group. AII protected against mortality from TBI

Treatment	Dose (µg/kg)	Day 3	Day 6	Day 9	Day 12	Day 15	Day 22
Placebo		100	100	100	85	70	0
AII, day 0	10	100	100	100	80	80	80
AII, day 0	100	100	100	100	100	100	100
AII, day 2	10	100	100	100	100	100	100
AII, day 2	100	100	100	100	100	100	100

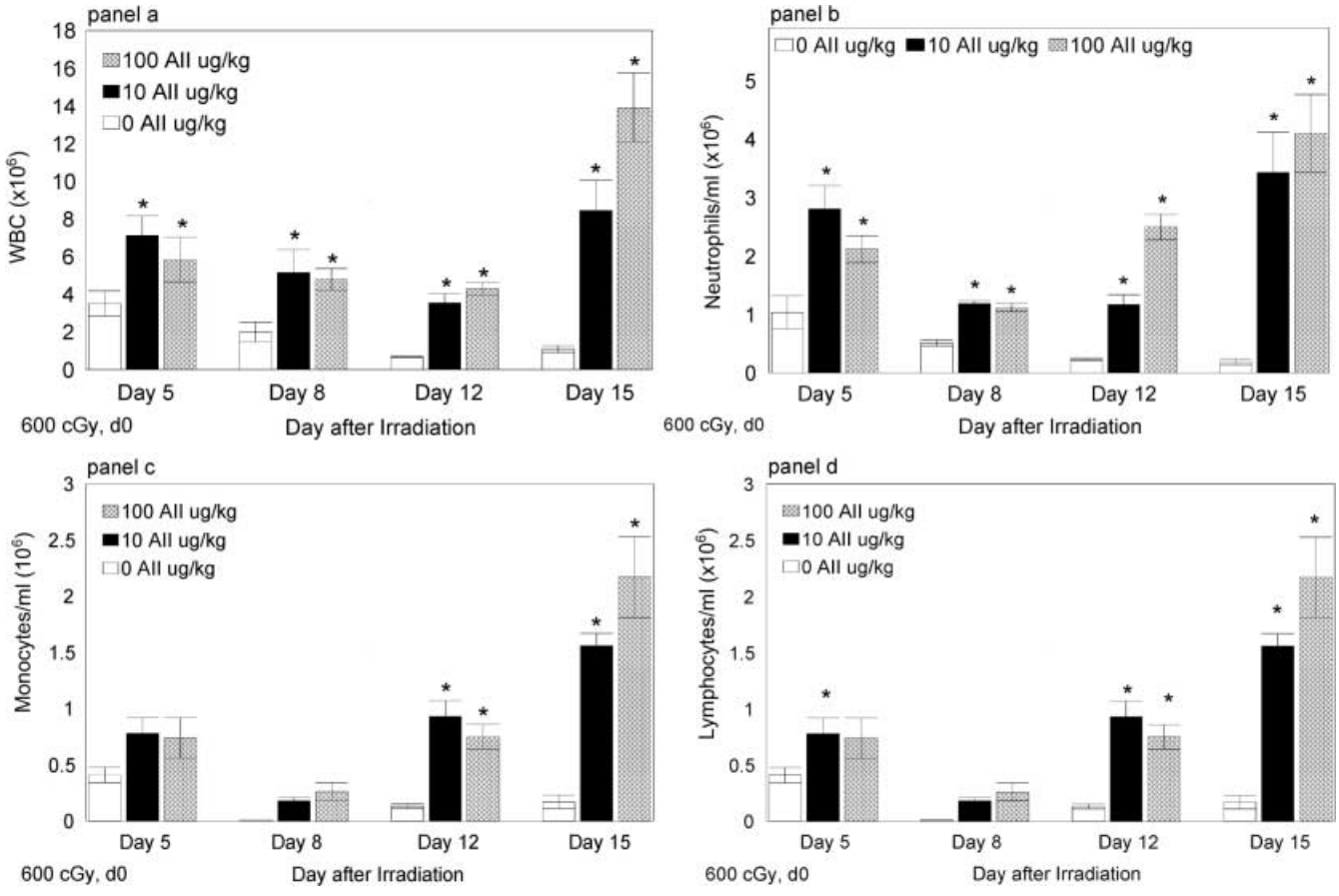


Fig. 1a–d. Effects of administration of AII on the numbers of circulating WBC (**a**), neutrophils (**b**), monocytes (**c**) and lymphocytes (**d**). The results presented represent the means ± SE from five animals per group. AII accelerated the recovery of peripheral WBC, neutrophils, monocytes and lymphocytes after TBI. $*P \leq 0.05$ vs control

and A(1–7) were shown to have increased GM-CFU, GEMM-CFU and BFU-E on day 14 after irradiation (Fig. 5).

Effect on megakaryocyte precursors and platelet concentration in the peripheral blood

The effect of administration of angiotensin peptides starting the day of irradiation on the number of megakaryocyte precursors in the bone marrow (CFU-Meg) was evaluated on day 14 after irradiation. Subcutaneous administration of AII or A(1–7) to irradiated animals resulted in approximately a tenfold increase in the level of megakaryocyte precursors over saline-treated irradiated controls (non-irradiated levels 5207 ± 1906) (Fig. 5). Further, the concentration of megakaryocytes in the bone marrow increased following administration of the peptide (Table 4).

The effect of angiotensin peptides on the number of platelets was also assessed at various times after irradiation. In the controls (no peptide treatment), the concentration of platelets progressively decreased throughout the postirradiation interval (Fig. 6). At all time-points, the animals treated with AII and A(1–7) starting on the day of irradiation had platelet counts at least 2.5-fold higher than the saline-treated controls.

Discussion

As was suggested by in vitro studies of the effect of AII on the proliferation of hematopoietic progenitors and

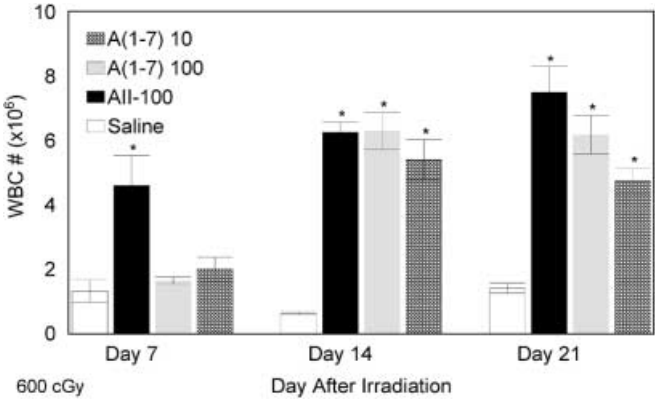


Fig. 2. Effects of administration of A(1–7) on the number of circulating WBC of irradiated recipients. The results presented represent the means ± SE from five animals per group. A(1–7) accelerated WBC recovery after TBI. $*P \leq 0.05$ vs control

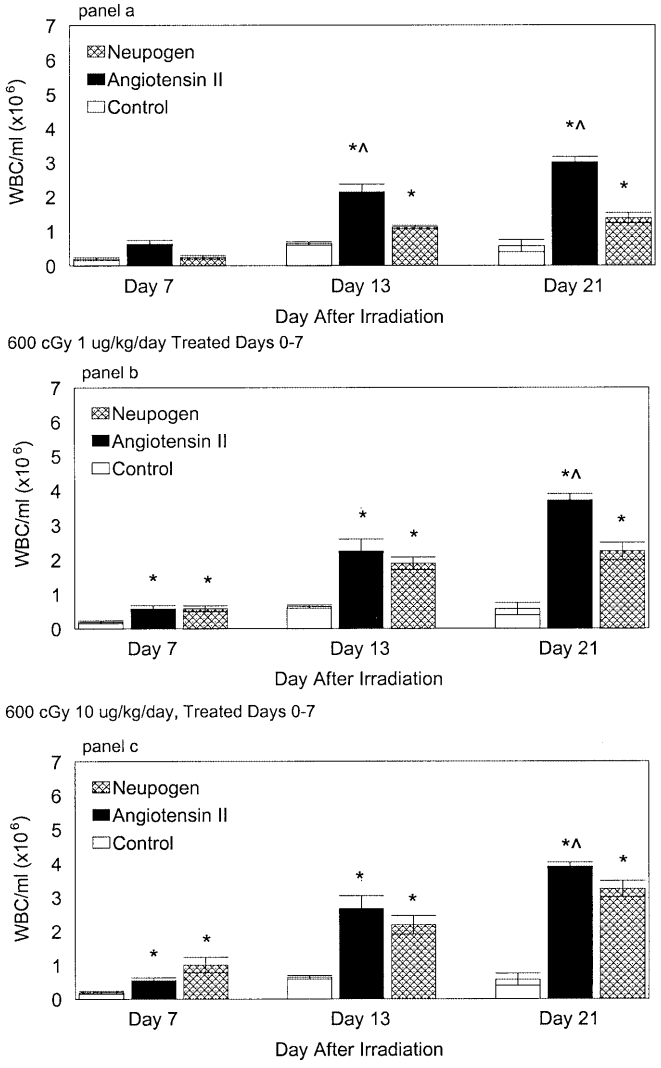


Fig. 3a–c. Effects of administration of AII or filgrastim on the day of irradiation and continuing until day 7 on the number of circulating WBC. The results presented represent the means ± SE from five animals per group. AII and filgrastim accelerated WBC recovery after TBI, but the effect of AII was more prolonged. *$P \leq 0.05$ vs control; ^$P \leq 0.05$ AII vs filgrastim

mesenchymal cells, hematopoietic recovery is accelerated after irradiation injury by administration of angiotensin peptides in vivo [37]. Animals treated with AII by subcutaneous injection beginning on day 0 or day 2 after irradiation, but not beginning 2 days prior to irradiation (day –2), were protected from bone marrow failure. Circulating WBCs, including multiple hematopoietic lineages (neutrophils, monocytes and lymphocytes), were restored to pretreatment levels. In addition, megakaryocytes were found in the peripheral blood in concentrations which increased with increasing dose and time. Further, A(1–7), a fragment of AII which does not induce hypertension, also increased the concentration of WBCs in the peripheral blood of irradiated mice. Irradiation studies comparing the effects of AII and filgrastim showed increases in peripheral blood WBC concentrations. However, the effect with AII treatment increased with time after irradiation. The relative difference between filgrastim and AII was more pronounced at lower doses, suggesting a more shallow dose-response curve with angiotensin peptides.

The potential for AII to stimulate the proliferation of hematopoietic progenitors through a receptor-mediated event has been reported previously [37]. An increase in the number of colonies formed by $CD34^+CD38^-$ cells from human cord blood and the expression of mRNA for AII receptors on $CD34^+CD38^-$ bone marrow cells is consistent with an angiotensin peptide-responsive effect early in hematopoiesis. Further support for these observations is provided in the current studies: (1) AII was also found to have caused an increase in the number of GM-CFU in bone marrow compared with placebo on day 35 whereas filgrastim had not; and (2) increases in the concentrations of GM-CFU, GEMM-CFU and BFU-E were observed in the bone marrow of irradiated animals treated with either AII or A(1–7). This suggests that angiotensin peptides do not act simply as survival factors but rather directly stimulate early progenitors to increase the progenitor pool within the bone marrow. This is further supported by similar results of AII on hematopoietic recovery after treatment with 5-fluorouracil, which is not a stem cell toxin (Rodgers et al., manuscript in preparation). In contrast to the absence of

Table 2. Effects on lymphocytes and neutrophils after 600 cGy TBI

Treatment	Lymphocytes ($\times 10^6$/ml)			Neutrophils ($\times 10^6$/ml)		
	Day 7	Day 13	Day 21	Day 7	Day 13	Day 21
Control	0.02 ± 0.01	0.36 ± 0.02	0.14 ± 0.05	0.11 ± 0.02	0.14 ± 0.02	0.39 ± 0.12
Angiotensin II (μg/kg per day)[a]						
1	0.23 ± 0.05	1.06 ± 0.33	1.08 ± 0.11	0.35 ± 0.01	0.28 ± 0.11	1.51 ± 0.31
10	0.17 ± 0.03	1.62 ± 0.22	2.11 ± 0.18	0.43 ± 0.09	0.37 ± 0.13	1.23 ± 0.09
100	0.28 ± 0.25	1.27 ± 0.52	1.85 ± 0.17	0.31 ± 0.06	0.80 ± 0.05	1.68 ± 0.23
Filgrastim (μg/kg per day)						
1	0.12 ± 0.03	0.64 ± 0.10	0.74 ± 0.03	0.11 ± 0.02	0.36 ± 0.21	0.68 ± 0.07
10	0.15 ± 0.03	0.93 ± 0.10	1.06 ± 0.04	0.49 ± 0.05	0.57 ± 0.08	0.69 ± 0.23
100	0.14 ± 0.05	0.36 ± 0.01	0.65 ± 0.17	0.43 ± 0.14	0.69 ± 0.34	1.01 ± 0.30

[a]Drug administered days 0–7 after irradiation

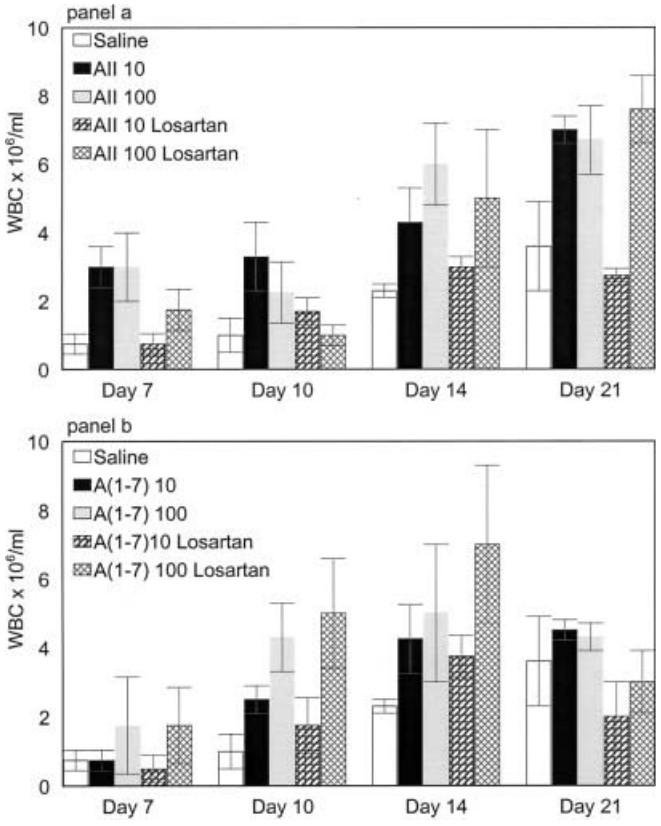


Fig. 4a, b. Effects of administration of AII and A(1–7) in the presence and absence of 50 μg/kg per day losartan on the number of circulating WBC of irradiated recipients. The results presented represent the means ± SE from five animals per group. Losartan blocked the recovery of WBC stimulated by AII, but did not affect the recovery stimulated by A(1–7)

benefit when AII was started prior to irradiation, AII stimulated hematopoietic recovery when administered either before or after chemotherapy. However, pretreatment with angiotensin peptides prior to cyclophosphamide, a stem cell toxin (as is irradiation), has been found to result in diminished hematopoietic recovery (unpublished observations).

Previous studies have suggested that recovery of hematopoiesis associated with angiotensin peptide therapy after myelosuppression is receptor mediated. Studies have shown that mRNA for AII type 1 receptors is expressed on $CD34^+CD38^-$ cells, $CD34^+CD38^+$ cells and lymphocytes [37]. Platelets also express angiotensin receptors [1, 9, 43]. Therefore, angiotensin peptides may act directly on the early progenitor cells as well as indirectly through the release of growth factors, to accelerate hematopoietic recovery. This is supported by the observation that losartan, an antagonist of the AT_1 receptor, blocked the acceleration in WBC recovery after irradiation. However, losartan had no effect on the recovery of WBCs after irradiation when the animals were also treated with A(1–7). Previous studies have shown that AII and A(1–7) can mediate similar effects through different receptors [25, 32, 46]. Potential

Table 3. Effects of AII and filgrastim on the number of myeloid progenitors in the bone marrow on day 35 after irradiation, The values are the mean ± SE of the results from five animals per group. Four weeks after cessation of treatment, the effect of AII on bone marrow progenitors continued to be significant

Treatment	Dose (μg/kg)	CFU-GM/femur
Placebo		3,790 ± 640
AII	1	11,740 ± 1,640*
	10	11,600 ± 1,470*
	100	13,100 ± 1,600*
Filgrastim	1	5,700 ± 750
	10	6,400 ± 500
	100	5,700 ± 310

*$P \leq 0.05$ vs control

receptors for the action of A(1–7) include the type 2 and type 7 receptors.

Colony-stimulating factors (CSFs) are naturally occurring glycoproteins that increase the production and maturation of hematopoietic precursors. G-CSF, or filgrastim, is a recombinant cytokine currently used in clinical practice to increase neutrophil levels in neutropenic patients [6, 10, 12, 29, 43, 45]. G-CSF stimulates the number of granulocytes in the peripheral circulation of neutropenic or immune-compromised hosts by facilitating mobilization from the marrow. In clinical trials, G-CSF has been shown to decrease infectious morbidity in compromised oncology and transplantation patients [16, 19, 42]. In the mouse model of irradiated bone marrow injury used in this study, AII was shown to be comparable to filgrastim in its ability to enhance circulating WBC recovery at early time-points. At later time-points, AII-treated animals had higher concentrations of circulating WBCs than their filgrastim-treated counterparts. However, in contrast with filgrastim which is limited to neutrophil response, the effect observed with angiotensin peptides was on WBCs from multiple hematopoietic lineages.

G-CSF has been shown to reduce antibiotic use and the number of febrile neutropenic episodes in clinical trials. This may be due to the ability of G-CSF to stimulate the number, differentiation and function of neutrophils, typically the first leukocytes to appear at the site of infection. Also, these cells play a role in the initial clearance of a bacterial infection. Administration of AII has been shown to reduce abscess formation in an intraperitoneal infection potentiation model whereas filgrastim does not [38]. Similar results have been observed in this model with A(1–7) (unpublished observations). As angiotensin peptides increased neutrophil numbers in irradiated animals, the benefits of this peptide in compromised hosts may be both directly and indirectly associated with a variety of responses to injury. Angiotensin peptides increased the number of platelets in the peripheral blood and the number of myeloid progenitors in the bone marrow after irradiation. Because filgrastim acts on myeloid precursor cells to stimulate granulocyte production without stimulating the formation of new myeloid progenitors, whereas angiotensin peptides seem to act at both sites, angiotensin

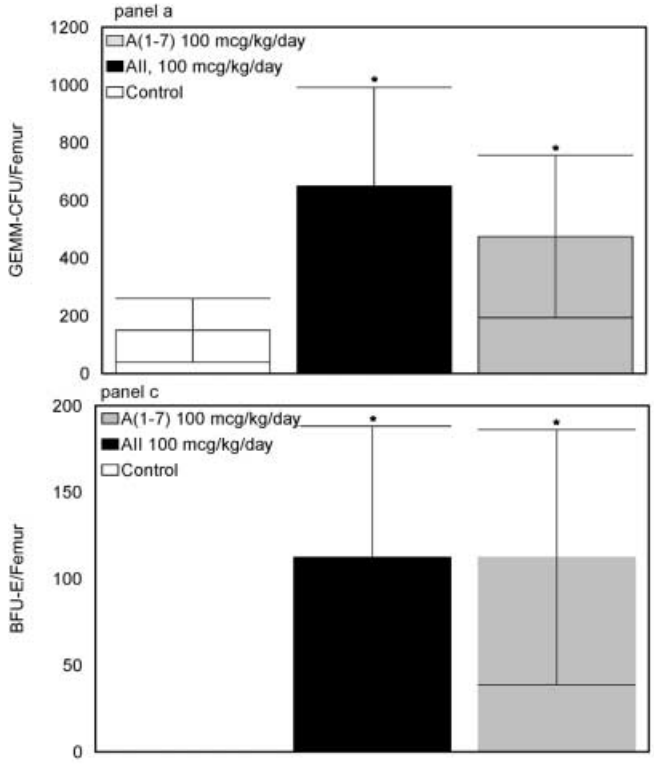
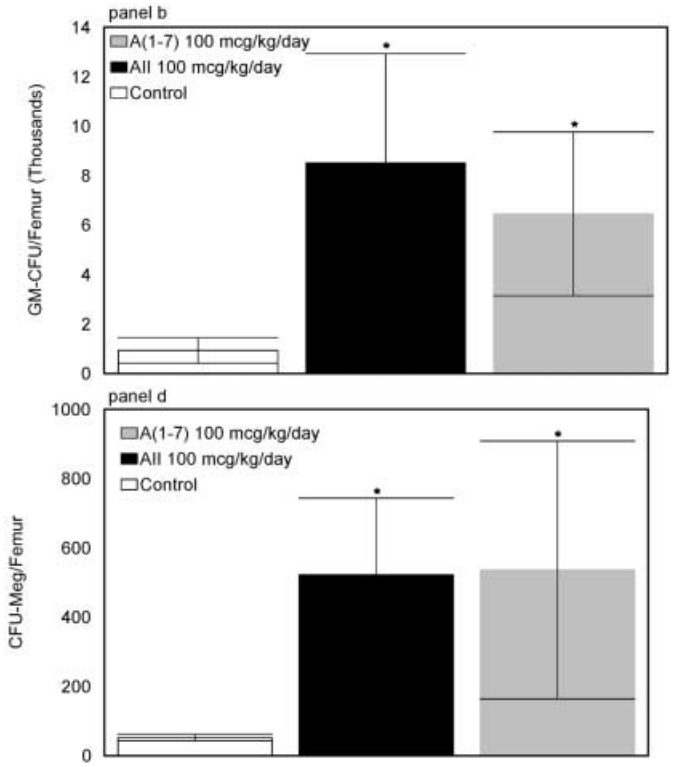


Fig. 5a–d. Effects of administration of AII and A(1–7) starting 2 days after irradiation and continuing until day 14 on the number of myeloid (**a**, **b**), erythroid (**c**) and megakaryocyte (**d**) progenitors. The results presented represent the means ± SE from five animals per group. Both AII and A(1–7) increased the number of progenitors of multiple lineages in the bone marrow. *$P \leq 0.05$ vs control

Table 4. Effect of angiotensin peptides on megakaryocyte number in the bone marrow. AII and A(1–7) increased the number of megakaryocytes in the bone marrow of irradiated mice

Treatment	Dose (μg/kg)	Megakaryocytes/femur
Placebo	0	25,000 ± 3,000
AII	100	71,000 ± 12,400*
A(1–7)	10	111,000 ± 27,000*
	100	95,000 ± 45,000*

*$P \leq 0.05$ vs control



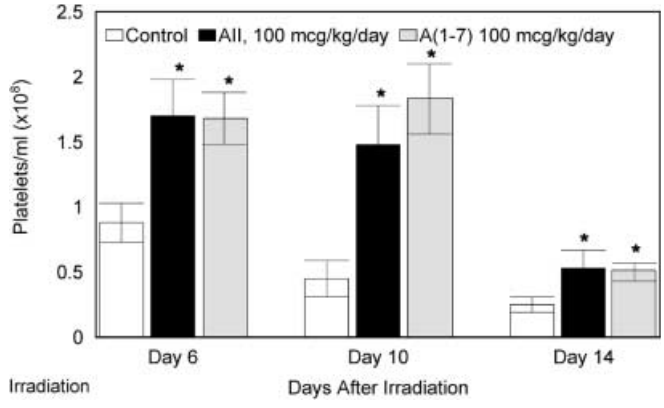


Fig. 6. Effects of administration of AII and A(1–7) on the number of circulating platelets of irradiated recipients. The results presented represent the means ± SE from four animals per group. AII and A(1–7) reduced the nadir observed with TBI. *$P \leq 0.05$ vs control

peptides may continue to facilitate hematopoietic recovery throughout multiple courses of chemotherapy. This hypothesis is supported by recent clinical trial results in breast cancer patients receiving three cycles of chemotherapy [5]. If subsequent chemotherapy therapy could be timed such that the effect of angiotensin peptides on progenitor proliferation had ceased, then subsequent administration of angiotensin peptides should continue to provide a therapeutic response through multiple courses of irradiation or chemotherapy.

In summary, systemic administration of angiotensin peptides led to an acceleration in hematopoietic recovery after irradiation. Further, these peptides acted to stimulate the formation of bone marrow progenitors used to effect recovery of mature elements after myelosuppressive irradiation.

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