Hematopoiesis in 5-Fluorouracil-treated Adenosine A_3 Receptor Knock-out Mice

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Short title: Hematopoiesis in adenosine A_3 receptor knock-out mice

Summary

The purpose of the study was to describe and compare normal and 5-fluorouracil (5-FU)-suppressed hematopoiesis in adenosine A_3 receptor knock-out (A_3AR KO) mice and their wild-type (WT) counterparts. To meet the purpose, a complex hematological analysis comprising nineteen peripheral blood and bone marrow parameters was performed in the mice. Defects previously observed in the peripheral blood erythrocyte and thrombocyte parameters of the A_3AR KO mice were confirmed. Compartments of the bone marrow progenitor cells for granulocytes/macrophages and erythrocytes were enhanced in the control, as well as in the 5-FU-administered A_3AR KO mice. 5-FU-induced hematopoietic suppression, evaluated on day 2 after the administration of the cytotoxic drug, was found to be significantly deeper in the A_3AR KO mice compared with their WT counterparts, as measured at the level of the bone marrow progenitor cells. The rate of regeneration, as assessed between days 2 and 7 after 5-FU administration, was observed in the population of the granulocyte/macrophage progenitor cells to be higher in the A_3AR KO mice in comparison with the WT ones. The increased depth of 5-FU-induced suppression in the compartments of the hematopoietic progenitor cells in the A_3AR KO mice represents probably a hitherto undescribed further consequence of the lack of adenosine A_3 receptors and indicates its synergism with the pharmacologically induced cytotoxic action of 5-FU.

Key words

Adenosine A_3 receptor knock-out mice; hematopoiesis; 5-fluorouracil-induced hematotoxicity

Introduction

Adenosine A_3 receptors belong to the family of adenosine receptors that are integral membrane molecules mediating cell signaling by their purinergic ligands (e.g., Abbracchio and Burnstock 1998, Poulsen and Quinn 1998, Fredholm *et al.* 2001, Klotz 2000). Signaling through adenosine receptors was found to modulate cell proliferation, differentiation, and apoptosis (Jacobson *et al.* 1999, Schulte and Fredholm 2003).

The hematopoiesis-regulating role of adenosine receptors in general and adenosine A_3 receptors in particular under the conditions of their pharmacological activation has been intensively investigated by the authors (for review see Hofer and Pospíšil 2006, Hofer *et al.* 2011). Briefly summarized, adenosine receptors can be pharmacologically activated either non-selectively, by their natural agonist adenosine, or selectively, by synthetic adenosine analogs (Jacobson 2002). We have found that non-selective activation of adenosine receptors stimulates hematopoiesis in normal and ionizing radiation-exposed mice (e.g., Pospíšil *et al.* 1992, 1993, Hofer *et al.* 1995, 1997). The ability of non-selective activation of adenosine receptors to up-regulate hematopoiesis has been shown also in conditions of hematopoietic suppression induced by the cytotoxic drug 5-fluorouracil (e.g., Hofer *et al.* 2001).

In subsequent studies, selective stimulation of adenosine A_3 receptors by their selective agonist N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (IB-MECA) was found to be responsible for the previously observed stimulatory action of non-selective adenosine receptor stimulation: IB-MECA was found to stimulate proliferation of hematopoietic progenitor cells (Pospíšil *et al.* 2004) and to support hematopoietic regeneration following application of cytotoxic antitumor drugs (Merimsky *et al.* 2003, Hofer *et al.* 2006) or ionizing radiation (Hofer *et al.* 2010).

Of interest were the results of a study using real-time PCR (RT-PCR) showing that adenosine A_3 receptors are expressed in four mouse hematopoietic precursor cells (Štreitová *et al.* 2010). The result points out that a direct stimulation of these cells with a selective adenosine A_3 receptor is possible. Another RT-PCR study revealed that the expression of mRNA for adenosine A_3 receptors on model promyelocytic HL-60 cells is dependent on the cell cycle phases (Hofer *et al.* 2011). This finding enables us to formulate hypotheses on the mechanisms of the regulatory action of adenosine A_3 receptors in hematopoiesis. The studies on the expression of adenosine A_3 receptors in hematopoietic cells bear connection to the latest investigations summarized below.

Most recently a new insight into the topic of the regulation of hematopoiesis through adenosine A_3 receptors has been made possible by the utilization of adenosine A_3 receptor knock-out (A_3AR KO) mice. The description and analysis of the state of hematopoiesis in individual cell compartments of the peripheral blood and the bone marrow has enabled us to reveal cell populations affected by the lack of adenosine A_3 receptors. Thus, defects have been described in the populations of the mouse peripheral blood erythrocytes and platelets of the A_3AR KO mice (Hofer *et al.* 2013a). The succeeding studies comprising also exposition of A3AR KO mice to ionizing radiation have revealed that the defects at the level of mature peripheral blood cells are attempted to be compensated from the level of the bone marrow progenitor cells (Hofer *et al.* 2014a).

The results obtained in this communication extend the knowledge on the functioning of adenosine A_3 receptors in hematopoiesis. A_3AR KO mice were experimentally exposed to 5-fluorouracil and the kinetics of the induced hematological damage and the subsequent regeneration were followed.

Material and Methods

Mice

Adenosine A_3 receptor knock-out (Adora^{tm1jbsn}/Adora^{tm1Jsbn}, A_3AR KO) male mice, backcrossed onto a C57BL/6 background (Salvatore *et al.* 2000), were obtained from Merck Research Laboratories (West Point, PA, USA) and bred in the Laboratory Animal Breeding and Experimental Facility of the Faculty of Medicine, Masaryk University, Brno, Czech Republic. Wild-type (WT) C57BL/6 mice were obtained from the Laboratory Animal Breeding and Experimental Facility of the Faculty of Medicine, Masaryk University, Brno, Czech Republic. For material sampling 2.5 months old mice were used.

5-fluorouracil (5-FU) administration, sampling of material

5-fluorouracil (5-FU) (Sigma, St. Louis, MO, USA) was dissolved in saline and given intraperitoneally (i.p.) in a single dose of 100 mg/kg in a volume of 0.2 ml. This dose was based on previous experimental experience (Weiterová *et al.* 2000). Sampling of material was performed on days 2 and 7 after 5-FU administration, and in untreated control mice.

Hematological techniques

For evaluation of the peripheral blood parameters, the animals were anesthetized with an i.p. injection of 0.07 ml of Narkamon (ketamine in the form of ketamine hydrochloride)/Rometar (xylazine in the form of xylazine hydrochloride) solution (5% Narkamon and 2% Rometar [both Spofa, Praha, Czech Republic]) in a ratio of 2.63:1, and the peripheral blood was sampled by cardiac puncture. The numbers of total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, erythrocytes, and platelets per 1 µl of the peripheral blood were determined by an Auto Hematology Analyzer Mindray 5300Vet (Shenzen, China). The same device was used for the determination of blood hemoglobin level

(HGB), hematocrit (HCT), mean erythrocyte volume (MCV), mean erythrocyte hemoglobin (MCH), mean erythrocyte hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW).

In mice sacrificed by cervical dislocation, the femurs were removed, marrow cells were harvested by standard procedures, and the numbers of nucleated cells of the femoral bone marrow were determined using a Coulter Counter (Model ZF, Coulter Electronics, Luton, UK). Standard procedures were used for *in vitro* assays of the femoral clonogenic progenitor cells. Granulocyte-macrophage colony-forming cells (GM-CFC) were assessed using the MethoCult M3001 medium (StemCell Technologies, Vancouver, Canada). Erythroid progenitor cells (BFU-E) were determined using the MethoCult SF M3436 medium (Stem Cell Technologies). Femoral marrow cell suspensions were plated (1.5×10^5 and 1×10^5 nucleated bone marrow cells for GM-CFC and BFU-E, respectively) in triplicate for both assays and incubated at $37\ ^0$C in a humidified atmosphere containing 95% air and 5% CO_2. GM-CFC were scored after a 7-day incubation as colonies containing 50 or more cells. Hemoglobinized colonies were counted as BFU-E after an 8-day incubation. Considering the differences in the average body weight between the groups of mice studied (22.5 g, 21.3 g, 21.6 g, 24.6 g, 23.3 g, and 22.8 g for untreated A_3AR KO mice, A_3AR KO mice on day 2 after administration of 5-FU, A_3AR KO mice on day 7 after administration of 5-FU, untreated WT mice, WT mice on day 2 after administration of 5-FU, and WT mice on day 7 after administration of 5-FU, respectively), the values of the bone marrow parameters were expressed as per 1 g of body weight.

Statistics

The values are presented as arithmetic means ± standard errors of the means (SEM). In each parameter, the global statistical significance among the groups was assessed using one-way ANOVA. The differences between the individual experimental groups were evaluated using the Mann-Whitney test. Each experiment was performed twice. The analyses were performed on data of combined experiments after their standardization using the z-score.

For the parameters of the numbers of neutrophils and erythrocytes per 1 µl of the peripheral blood, as well for those of the numbers of GM-CFC/femur and BFU/femur per 1 g of body weight, independent experimental series of untreated A_3AR KO mice + A_3AR KO mice on day 2 after the administration of 5-FU + A_3AR KO mice on day 7 after the administration of 5-FU, and that of untreated WT mice + WT mice on day 7 after the administration of 5-FU + WT mice on day 7 after the administration of 5-FU were recognized, in another analysis, as blocks. These blocks of combined data from A_3AR KO mice and WT mice were evaluated on the basis of two-way ANOVA models.

Two-way ANOVA models were also used for comparison of the rates of decrease from the control state (untreated mice) to the state in mice on day 2 after the administration of 5-FU, as well as of differences in the rates of increase from the state in mice on day 2 after the administration of 5-FU and that in mice on day 7 after the administration of 5-FU between A_3AR KO mice and WT mice. These statistical analyses concerned nine selected hematological parameters (see Results).

Results

Peripheral blood leukocyte parameters

The summary of findings in the peripheral blood leukocyte parameters in the A_3AR KO mice and their WT counterparts is presented in Table 1. Comparisons were performed between untreated A_3AR KO mice and WT mice, between A_3AR KO mice and WT mice on day 2 after the administration of 5-FU, and between these two groups of mice on day 7 after the administration of 5-FU. With the exception of one comparison in the blood eosinophil count, no significant differences were observed between the A_3AR KO mice and the WT mice. Since the numbers of blood neutrophils were observed to be always lower in the A_3AR KO mice, the approach of comparison of their values between A_3AR KO mice and WT mice, taken as blocks (see Material and Methods), was used. However, the P value reached not even under these conditions a statistical significance (0.098).

Peripheral blood erythrocyte parameters

The peripheral blood erythrocyte parameters are summarized in Table 2. The blood erythrocyte count was found to be statistically higher in untreated A_3AR KO mice in comparison with their WT counterparts. Since also on days 2 and 7 after the administration of 5-FU the numbers of erythrocytes were higher in the A_3AR KO mice than in the WT ones, the approach of comparing these mice as blocks was used again. In this approach, the erythrocyte counts were found to be, in general, significantly higher in the A_3AR KO mice ($P = 0.006$). On the other hand, significantly lower values of MCV and MCH were observed in the A_3AR KO mice in all comparisons (untreated mice, mice on day 3 after the administration of 5-FU, and mice on day 7 after the administration of 5-FU).

Peripheral blood platelet parameters

Parameters concerning the peripheral blood platelets are shown in Table 3. Generally, a decline in the platelet parameters in the A_3AR KO mice can be stated. While the blood

platelet count in these mice was found to be significantly lower in one comparison (day 2 after the administration of 5-FU), the parameters of MPV and PDW were significantly decreased in all three comparisons, and that of PCT in untreated mice and on day 2 after the administration of 5-FU.

Bone marrow parameters

On the other hand, an activation of hematopoiesis, as assessed by the femoral bone marrow parameters (Table 4) was observed. The femoral bone marrow cellularity was observed to be significantly higher in untreated A_3AR KO mice. In the A_3AR KO mice, the numbers of femoral granulocyte-macrophage progenitor cells (GM-CFC) were found to be significantly higher on day 7 after the administration of 5-FU and those of erythroid progenitor cells (BFU-E) in untreated mice. The approach of block comparison of the A_3AR KO mice and their WT counterparts in the parameters of femoral GM-CFC and BFU-E revealed significantly higher values in the knock-out mice ($P = 0.001$ and $P < 0.001$, respectively).

Comparison of the 5-FU-induced damage and subsequent regeneration between A_3AR KO mice and their WT counterparts

The magnitude of 5-FU-induced damage to selected hematopoietic parameters (neutrophil, lymphocyte, monocyte, eosinophil, erythrocyte, and thrombocyte peripheral blood counts, femoral bone marrow cellularity, and the numbers of femoral GM-CFC and BFU-E) was evaluated as the rate of decrease between the values of these parameters in untreated mice and those found on day 2 after the administration of 5-FU. The ability of the mice to regenerate from the 5-FU-induced damage was assessed as the rate of increase between the values found on days 2 and 7 after the administration of 5-FU. The rates of decrease or increase were

calculated as ratios of the values between which the decreases or increases take part. Significant differences in this indicator between the A_3AR KO mice and their WT counterparts were obtained nearly exclusively for the parameters of the femoral marrow progenitor cells. A significantly higher rate of decrease between the control state and day 2 after the administration of 5-FU was found in the A_3AR KO mice both for the femoral GM-CFC and BFU-E ($P = 0.01$, $P = 0.004$, respectively). The rate of increase between the states on days 2 and 7 was calculated to be significantly higher in the A_3 AR KO mice in the parameter of the femoral GM-CFC ($P = 0.037$). Besides these findings in the marrow progenitor cell compartments only one more significantly different rate of increase between days 2 and 7 was found, namely in the parameter of the blood platelet count, where the rate of increase was significantly higher in the A_3AR KO mice ($P = 0.022$).

Discussion

The findings in the peripheral blood parameters have confirmed previous observations (Hofer *et al.* 2013a, 2014a) on the existence of defects in functional properties of erythrocytes and thrombocytes in the A_3AR KO mice, as seen from the parameters of the mean erythrocyte volume, mean erythrocyte hemoglobin, and mean platelet volume (see Tables 2 and 3). Whether these particular findings reflect also in an aggravation of the parameters like oxygen transport capacity, arterial pO_2, or platelet aggregation test, an additional experimentation is, however, needed. An also previously described (Hofer *et al.* 2014a) obvious increase in the numbers of the bone marrow hematopoietic progenitor cells, GM-CFC and BFU-E, in the A_3AR KO mice, explainable as an attempt of the hematopoietic system of these mice to compensate for the peripheral blood cell insufficiency, was observed in this study (see Table 4). In the erythroid compartment, the probable compensatory activity in the A_3AR KO mice

was apparently successful, as follows from their significantly higher blood erythrocyte count and blood hemoglobin level (see Table 2).

A sufficient number of the A_3AR KO mice available for this study enabled to analyze the state of hematopoiesis in two time intervals following the administration of the cytotoxic drug, 5-FU, namely on days 2 and 7 after the 5-FU administration. These two time intervals can be designated as the time intervals of the states of damage and regeneration. The analyses performed for the processes of damage (decrease between control mice and those on day 2 after the administration of 5-FU) and regeneration (increase between days 2 and 7 after the administration of 5-FU) enabled us to compare the kinetics of these processes in the A_3AR KO mice and their WT counterparts. The results of these analyses have shown that the expectably most sensitive compartments of the bone marrow progenitor cells, GM-CFC and BFU-E, are significantly more affected in the A_3AR KO mice than in the WT mice, as follows from the significantly higher rate of decrease in these parameters in the KO mice. This finding suggests that there exists yet another hematopoietic defect caused by the lack of adenosine A_3 receptors than those hitherto observed in the peripheral blood cell parameters of the A_3AR KO mice. The mechanism of the origin of this defect responsible for a higher susceptibility of the hematopoietic progenitor cells to the 5-FU-induced damage remains to be investigated.

The studies performed on the hematopoiesis of the A_3AR KO mice so far have not comprised the parameter of the hematopoietic stem cells. However, recent investigations in normal mice have not demonstrated any efficacy of the administration of IB-MECA, an adenosine A_3 receptor agonist on the hematopoietic stem cell compartment (Hofer *et al.* 2013b).

A more general information about the overall health state of the A_3AR KO mice could be provided by survival studies. The up to now performed numerous experiments on the survival

of normal mice after lethal irradiation and an adenosine A_3 receptor agonist administration have revealed an efficacy of the therapeutic intervention only if the agonist was given very early after administration (Hofer *et al.* 2014b). Several other administration schedules of the agonist, including its repeated administration, did not reveal any efficacy on post-irradiation survival of experimental mice (Hofer *et al.* 2012). Since post-irradiation survival is closely associated with the overall hematological state, survival studies might provide a significant piece of information on the ability of the hematopoietic system of the A_3AR KO mice to cope with the lack of the adenosine A_3 receptor.

The adenosine A_3 receptor and its agonists have been in the midst of attention due to a number of indications including, e.g., those of cardiovascular (Hussain *et al.* 2014) or oncological diseases (Antonioli *et al.* 2013). Their involvement in the processes of hematopoiesis, as well as in hematological diseases and their therapy, has been rather neglected by the majority of laboratories dealing with this general topic. However, recent findings on the efficacy of adenosine receptor agonists in the modulation of hematopoiesis obtained predominantly by the authors highlight the significance of adenosine A_3 receptors in hematopoiesis and their possible employment in clinical practice. Investigations in the A_3AR KO mice represent a promising tool in the research on the mechanisms through which adenosine and further agonists of its receptors execute their roles in hematopoietic regulation processes.

Conflict of Interest

There is no conflict of interest.

Acknowledgments

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Table 1. Values of peripheral blood leukocyte parameters in untreated, 5-fluorouracil (5-FU)-treated (day 2 after treatment), and 5-FU-treated (day 7 after treatment) adenosine A_3 receptor knock-out (A_3AR KO) and wild-type (WT) mice

Parameter	Untreated A_3AR KO mice n = 6	Untreated WT mice n = 6	A_3AR KO mice – day 2 after 5-FU n = 9	WT mice – day 2 after 5-FU n = 9	A_3AR KO mice – day 7 after 5-FU n = 8	WT mice – day 7 after 5-FU n = 8
Blood leukocyte count (x 10^9/l)	4.8 ± 0.47	5.2 ± 0.35	4.2 ± 0.67	3.8 ± 0.21	1.9 ± 0.28	2.1 ± 0.17
Blood granulocyte count (x 10^9/l)	0.64 ± 0.084	0.74 ± 0.073	0.51 ± 0.070	0.61 ± 0.050	0.06 ± 0.014	0.10 ± 0.047
Blood lymphocyte count (x 10^9/l)	3.8 ± 0.45	4.1 ± 0.36	3.6 ± 0.62	3.2 ± 0.21	1.7 ± 0.25	1.9 ± 0.15
Blood monocyte count (x 10^9/l)	0.33 ± 0.044	0.37 ± 0.065	0.06 ± 0.018	0.06 ± 0.008	0.04 ± 0.006	0.01 ± 0.005
Blood eosinophil count (x 10^9/l)	0.05 ± 0.019	0.09 ± 0.039	0.06 ± 0.036	0.04 ± 0.023	0.01 ± 0.006 *	0.02 ± 0.007

The results are presented as arithmetic means ± standard errors of the means (SEM). n = numbers of mice. * - the value in A_3AR KO mice is statistically significantly ($P \leq 0.05$) lower than that in the corresponding WT counterparts.

Table 2. Values of peripheral blood erythrocyte parameters in untreated, 5-fluorouracil (5-FU)-treated (day 2 after treatment), and 5-FU-treated (day 7 after treatment) adenosine A_3 receptor knock-out (A_3AR KO) and wild-type (WT) mice

Parameter	Untreated A_3AR KO mice n = 6	Untreated WT mice n = 6	A_3AR KO mice – day 2 after 5-FU n = 9	WT mice – day 2 after 5-FU n = 9	A_3AR KO mice – day 7 after 5-FU n = 8	WT mice – day 7 after 5-FU n = 8
Blood erythrocyte count (x 10^{12}/l)	9.1 ± 0.15 ##	8.3 ± 0.12	8.2 ± 0.27	8.2 ± 0.15	6.5 ± 0.16	6.1 ± 0.18
Blood hemoglobin level (HGB) (g/l)	132 ± 2.3 #	125 ± 2.5	117 ± 4.8	122 ± 2.0	93 ± 2.6	91 ± 2.8
Hematocrit (HCT) (%)	45.1 ± 0.91	43.0 ± 0.87	39.7 ± 1.54	41.5 ± 0.78	31.1 ± 0.89	30.7 ± 0.92
Mean erythrocyte volume (MCV) (fl)	50.0 ± 0.36 **	51.8 ± 0.35	48.7 ± 0.29 ***	50.7 ± 0.25	48.0 ± 0.29 ***	50.9 ± 0.33
Mean erythrocyte hemoglobin (MCH) (pg)	14.6 ± 0.06 **	15.1 ± 0.10	14.4 ± 0.11 ***	14.9 ± 0.09	14.3 ± 0.08 ***	15.0 ± 0.10
Mean erythrocyte hemoglobin concentration (MCHC) (g/l)	293 ± 1.0 #	291 ± 0.5	295 ± 1.5	294 ± 1.0	299 ± 1.2	295 ± 1.4
Red cell distribution width (RDW) (%)	11.9 ± 0.18 **	13.0 ± 0.26	12.2 ± 0.34	12.4 ± 0.14	11.7 ± 0.26	12.1 ± 0.28

The results are presented as arithmetic means ± standard errors of the means (SEM). n = numbers of mice. **, *** - the value in A_3AR KO mice is statistically significantly lower ($P \leq 0.01$, $P \leq 0.001$, respectively) than that in the corresponding WT counterparts. #, ## - the value in A_3AR KO mice is statistically significantly higher ($P \leq 0.05$, $P \leq 0.01$, respectively) than that in the corresponding WT counterparts.

Table 3. Values of peripheral blood platelet parameters in untreated, 5-fluorouracil (5-FU)-treated (day 2 after treatment), and 5-FU-treated (day 7 after treatment) adenosine A_3 receptor knock-out (A_3AR KO) and wild-type (WT) mice

Parameter	Untreated A_3AR KO mice n = 6	Untreated WT mice n = 6	A_3AR KO mice – day 2 after 5-FU n = 9	WT mice – day 2 after 5-FU n = 9	A_3AR KO mice – day 7 after 5-FU n = 8	WT mice – day 7 after 5-FU n = 8
Blood platelet count (x 10^9/l)	987 ± 44.8	1060 ± 57.0	882 ± 52.9 **	1029 ± 26.8	667 ± 57.4	609 ± 79.1
Mean platelet volume (MPV) (fl)	4.8 ± 0.02 **	5.5 ± 0.05	4.9 ± 0.09 **	5.3 ± 0.04	5.7 ± 0.11 **	6.5 ± 0.13
Plateletcrit (PCT) (%)	0.48 ± 0.022 **	0.59 ± 0.032	0.43 ± 0.027 ***	0.54 ± 0.013	0.37 ± 0.030	0.40 ± 0.056
Platelet distribution width (PDW) (%)	14.6 ± 0.04 **	15.0 ± 0.03	14.6 ± 0.09 *	14,8 ± 0.04	15.3 ± 0.09 *	15.6 ± 0.08

The results are presented as arithmetic means ± standard errors of the means (SEM). n = numbers of mice. *, **, *** - the value in A_3AR KO mice is statistically significantly lower ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively) than that in the corresponding WT counterparts.

Table 4. Values of femoral bone marrow parameters in untreated, 5-fluorouracil (5-FU)-treated (day 2 after treatment), and 5-FU-treated (day 7 after treatment) adenosine A_3 receptor knock-out (A_3AR KO) and wild-type (WT) mice

Parameter	Untreated A_3AR KO mice n = 6	Untreated WT mice n = 6	A_3AR KO mice – day 2 after 5-FU n = 9	WT mice – day 2 after 5-FU n = 9	A_3AR KO mice – day 7 after 5-FU n = 8	WT mice – day 7 after 5-FU n = 8
Femoral bone marrow cellularity / g body weight (x 10^3)	1.42 ± 0.067 ##	1.08 ± 0.069	0.64 ± 0.039 ##	0.46 ± 0.037	0.45 ± 0.032	0.42 ± 0.075
GM-CFC / femur / g body weight	598 ± 67.5	426 ± 43.7	25.0 ± 4.23	19.7 ± 5.21	344 ± 33.0 #	230 ± 33.4
BFU-E / femur / g body weight	584 ± 79.5 ##	400 ± 25.7	42.1 ± 5.10	23.6 ± 5.58	188 ± 18.2	136 ± 20.7

The results are presented as arithmetic means ± standard errors of the means (SEM). n = numbers of mice. #, ## - value in A_3AR KO mice is statistically significantly higher ($P \leq 0.05$, $P \leq 0.01$, respectively) than that in the corresponding WT counterparts.