Comparative Medicine
Copyright 2023
by the American Association for Laboratory Animal Science

Vol 73, No 6
December 2023
Pages 439–445

Original Research

Effects of Exogenous Erythropoietin on Rabbit (*Oryctolagus cuniculus*) Hematological and Biochemical Parameters

Jessica K Levine,[1,*] Josilene Nascimento Seixas,[2] Jana M Ritter,[2] Amanda Y Liew,[3] and Cassandra M Tansey[1]

Rabbits can develop anemia due to serial phlebotomy or secondary to induced disease states. This study evaluated the effects of a single injection and three consecutive injections of erythropoietin in rabbits at 150 IU/kg and 1,000 IU/kg in order to determine whether these dosages produce a sustained increase in hematocrit. Analysis of CBC and chemistry parameters showed significant elevation in hematocrit one week after administration of 1,000 IU/kg erythropoietin for three consecutive days. These results indicate that this dosage regimen can increase hematocrit in apparently healthy, nonanemic rabbits for one week.

Abbreviations and acronyms: EPO, erythropoietin; M:E, myeloid to erythroid ratio

DOI: 10.30802/AALAS-CM-22-000107

Introduction

Rabbits are used for a variety of research purposes. One unusual use is to maintain colonies of blood-sucking arthropod species that do not efficiently feed using existing artificial blood membrane technologies and require a blood meal from a live donor. Using rabbits to feed arthropod colonies and for disease models or studies that require frequent blood collection may result in anemia that can lead to secondary complications such as lethargy, decreased food intake, and, in severe cases, tissue hypoxia and organ damage. IACUC policies limiting blood withdrawal volumes aim to forestall such complications, thereby protecting the health and welfare of the rabbits and the validity of the research results. In addition, the development of anemia could trigger removal of rabbits from the study before the designated endpoint, requiring the use of more rabbits over the course of the study to attain the desired statistical power and in that way conflicting with the principles of the 3Rs (reduction, refinement, and replacement). Preventing anemia in rabbits used in such models is vital for preserving their health and wellbeing. However, the use of erythropoietin to prevent anemia in rabbits has not been studied extensively.

Erythropoietin (EPO) is an endogenous hormone produced mainly by the kidneys in direct response to low pO_2 and thus indirectly to low hemoglobin levels. Binding of EPO to the cell surface of erythroid progenitor cells stimulates red blood cell production by promoting cell division and preventing apoptosis of these progenitor cells.[11,32] EPO is commercially available as recombinant human erythropoietin (Epogen, Procrit) or erythropoietin analog (Darbepoetin α) for clinical treatment of anemia in humans, primarily as related to chronic kidney disease or chemotherapy. In rabbits, EPO can be administered subcutaneously or intravenously. EPO has a half-life of approximately 3 h after intravenous administration in healthy rabbits, although the half-life is prolonged after nephrectomy or by chronic renal disease.[3] After subcutaneous administration of EPO, rabbit show peak plasma concentrations in 10 to 12 h with a mean half-life of 17 h.[38] While humans show apoptosis of the youngest population of erythrocytes and rapid return to baseline in red blood cell mass, hematocrit, and hemoglobin within one to 2 wk after discontinuation of EPO or hypoxic stress,[16,17] this effect has not been directly studied in rabbits.

Existing literature on EPO use in rabbits focuses on its effects in nonhematopoietic organs. Studies include effects on the central and peripheral nervous system,[8,33] ischemic injury,[23] neonatal prematurity,[34] hypertension,[18] and bone repair.[21,27] Although some studies report incidental findings of increased hemoglobin in EPO treated rabbits,[18,27] little information is available on the direct effects of EPO on rabbit complete blood count (CBC) and clinical serum chemistry parameters. In addition, published doses and dosing schedules recommended for using EPO as a treatment for anemia in rabbits vary widely.[4,25]

This study aimed to evaluate the changes in clinical chemistry and CBC parameters after administration of EPO in rabbits, and to determine whether any long-term cellular changes occur in hematopoietic organs. Doses and dosing schedules were based on existing formulary recommendations for rabbits and on regimens used to treat anemia in human neonates; various doses and dosing schedules are used in patients at risk of physiologic or iatrogenic anemia (doses range from 50 to 5,000 IU/kg and dosing intervals range from one single dose to multiple doses per week).[4,8,12,18,20,21,23,25,27,29,33,34] We measured CBC and chemistry

Submitted: 21 Oct 2022. Revision requested: 09 Dec 2022. Accepted: 12 Dec 2022.
[1]Comparative Medicine Branch, Division of Scientific Resources; [2]Infectious Disease Pathology Branch, Division of High-Consequence Pathogens and Pathology; and [3]National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia
**Corresponding author. Email: qnc6@cdc.gov*

parameters in apparently healthy rabbits that received EPO to test the hypothesis that all doses of EPO would increase hematocrit and hemoglobin, with greater effects at higher doses and after multiple doses. At the end of the study, histopathology was used to assess the presence of injection site reactions, cellular changes to hematopoietic organs, or evidence of adverse effects related to EPO dosing, such as thromboembolism.[30] We expected no differences in histopathology between groups based on the timeline of dosing and the recovery interval provided prior to necropsy. To our knowledge, no prior literature has reported effects of variable EPO dosing in healthy New Zealand white rabbits or established an appropriate dose range and schedule of EPO for mitigation of study-related anemia in rabbits.

Materials and Methods

Animals. Healthy, intact New Zealand white rabbits (Charles River Laboratories [Saint Constant, QC, Canada]); *n* = 18 [5 males and 13 females], age one to 6 y, average weight 4.1 kg, weight range 3.7 to 4.7 kg) were used for this study. Eleven of the 18 rabbits were housed at the facility for use on a training protocol. Seven rabbits were purchased for use on a tick feeding protocol earlier in the year. These 7 rabbits were seronegative for tickborne diseases, had normal hematocrit at the conclusion of the tick feeding protocol, were transferred to the training protocol and underwent a minimum 3-mo washout period prior to use on this study. Sex and age distribution varied due to the availability of rabbits at the time of study, but is representative of the variation that may occur in research. Rabbits were housed in static cages (Techniplast R-suite, West Chester, PA) or floor housed in custom pens (Corners Limited, Kalamazoo, MI) with crinkle paper bedding (Shepherd Specialty Papers Enviro-dri, Framingham, MA) in a climate-controlled indoor pen (T = 61 to 72 °F [16 to 22 °C], humidity = 30 to 70%) and were maintained and handled in accordance with the *Guide for the Care and Use of Laboratory Animals*[9] and the institute's animal care policies, with a 12:12-h light:dark cycle (0600 to 1800). Rabbits were housed singly (*n* = 10) or in pre-existing established pairs (*n* = 8). One pair was moved to floor pen housing for 2 wk during the study; all other rabbits remained in static cages for the duration of the study. Rabbits were fed a commercially pelleted diet (LabDiet 5326 – Laboratory Rabbit Diet HF, Arden Hills, MN), Timothy hay, and fresh vegetable enrichment and were provided with reverse-osmosis (RO) water ad libitum. Vendor health screening confirmed rabbits were free from rabbit hemorrhagic disease virus (RHDV), rabbit rotavirus, adenovirus, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Salmonella* spp., *Lawsonia intracellularis*, *Treponema paraluiscuniculi*, *Clostridium piliforme*, *Francisella tularensis*, *Eimeria* spp., *Encephalitozoon cuniculi*, *Demodex*, *Cheyletiella*, *Sarcoptes scabei*, *Psoroptes cuniculi*, and tickborne disease. All rabbits were assessed twice daily for general appearance, food and water consumption, activity level, and fecal and urine production. All animal use was performed under an IACUC-approved protocol at an AAALAC International–accredited institution. One rabbit showed gross evidence of bronchopneumonia at necropsy, later confirmed by histopathology, but did not show any clinical signs during the study.

Experimental procedure. Rabbits were assigned to a control group (*n* = 2) or one of 4 experimental groups (*n* = 4 each) and received subcutaneous injections (either a single dose or 3 consecutive doses at 24 h intervals. Rabbits were assigned to treatment groups that had the same distributions of sex and age. Pair-housed rabbits were assigned to the same treatment group. Rabbits A1 and A2 (controls) received 0.3 mL of saline subcutaneously (equivalent volume to the lowest EPO dose group) as a single dose (*n* = 1) or 3 doses (*n* = 1), respectively, to mimic the dose regimen for the experimental groups. Group B1 received a single 150-IU/kg dose of EPO. Group B2 received 3 doses of 150 IU/kg EPO. Group D1 received a single 1,000-IU/kg dose of EPO. Group D2 received 3 doses of 1,000 IU/kg EPO. Rabbits were weighed on the first day of the study for dose volume calculations. Subcutaneous injections were administered in the dorsal thoracolumbar region 1 to 2.5 cm lateral to midline. Injection volumes for the 150-IU/kg dose ranged from 0.26 to 0.36 mL. Injection volumes for the 1,000-IU/kg dose ranged from 1.8 to 2.2 mL. Injections greater than 1.5 mL were given in two separate subcutaneous locations and injection sites were alternated each day to minimize injection-associated tissue trauma. All study activities were performed in the morning, with injections and blood collections done between 0800 and 1000.

Rabbits were handled by trained veterinarians, veterinary technicians, or animal care technicians. Venipuncture was performed by veterinarians and veterinary technicians. Rabbits were secured in restraint boxes (Plas Labs, Lansing, MI) for all blood collection. Topical lidocaine/prilocaine cream (HI-TECH pharmacal, Amityville, NY) was applied liberally to the ears of the rabbit and allowed to take effect for approximately 10 min prior to venipuncture. Wintergreen oil (Wintergreen oil, NF, Humco, Austin TX) was applied topically over the ear veins for its vasodilation properties. Approximately 3 mL of blood (approximately 1.3% total blood volume) was drawn from marginal ear veins and distributed between 3-mL BD K2 EDTA tubes and 3-mL BD SST (Fischer Scientific, Pittsburgh, PA) vacuum phlebotomy tubes for CBC and chemistry, respectively. The manufacturer does not specify a minimum fill volume for BD vacuum phlebotomy tubes; however, a 2010 study showed no effects on CBC parameters with 25% filling as compared with complete filling of BD tubes.[37] If an adequate volume of blood could not be collected from the ear veins, the central ear artery was used. While there is very little veterinary literature comparing arterial and venous blood values, a recent study has shown comparable biochemical parameters in rabbits.[36] Hemostasis was achieved via direct gentle pressure with gauze. Baseline bloodwork was drawn on day 0, followed by initial dosing. Follow-up blood was collected on days 7, 14, 21, and 28 after initial experimental dosing. Samples for CBC and serum chemistry were sent to an external laboratory (Antech Diagnostics, Smyrna GA). Inhouse hematocrit for all groups was evaluated using microhematocrit tubes (Jorgenson Laboratories, Loveland, CO). On day 28, rabbits received 0.25 mg/kg acepromazine (VET ONE, Boise, ID) IM for light sedation and vasodilation due to difficult venipuncture from repeated weekly phlebotomy.

At the conclusion of the study, both of the control rabbits (A1 and A2) and 2 rabbits from groups B1, B2, D1, and D2 were euthanized for gross necropsy and histopathology. Anesthesia was induced with 15 mg/kg ketamine (Zetamine Injection, Akorn, Lake Forest, IL) IM and 1 mg/kg acepromazine IM and maintained via nose cone with isoflurane at 5% to ensure adequate anesthetic depth prior to euthanasia with intravenous pentobarbital/phenytoin (Beuthanasia-D, Akorn, Lake Forest, IL).

Pathology. Necropsy was performed by veterinary residents and a veterinary pathologist immediately after euthanasia; organs were evaluated grossly in situ and then removed. Splenic measurements and weights were obtained. Bone marrow Romanowsky-stained smears were prepared for cytologic evaluation. A small bone fragment from the femur with intact marrow was collected for histopathologic evaluation. After fixation, the marrow was carefully separated from the bone. Samples from hematopoietic organs (spleen and bone marrow), as well as kidney,

heart, lungs, CNS, liver, and GI tract, were fixed in 10% neutral buffered formalin, processed for routine paraffin histology, sectioned at 4 µm, and stained by hematoxylin and eosin (H and E) or Prussian blue iron stain. Pathologists performing the necropsy and histologic evaluation were blind to experimental groups.

Two veterinary pathologists each evaluated all tissues for inflammatory, degenerative, or other significant histopathologic changes. Evaluation of the cytologic and histologic preparations of bone marrow included assessment of bone marrow cellularity, iron stores, megakaryocyte count, myeloid to erythroid (M:E) ratio, and proportions of cells at various stages of erythroid and myeloid maturation.

Histologic evaluation of the bone marrow was performed using semiquantitative assessment of: 1) cellularity via visual estimation at low magnification (5×) of the percentage of marrow area occupied by cells compared with fat, with values less than 25% considered low cellularity, values between 25% and 75% considered normal cellularity, and values above 75% considered high cellularity; 2) megakaryocyte count and maturation: the number of megakaryocytes in 5 high-power (40×) fields were counted and reported as the mean number of megakaryocyte per 5 high-power fields; a range of 2.1 to 7.7 (mean = 4.6) is seen in healthy rabbits.[26] Maturation of megakaryocytes was considered normal if > 50% were mature, characterized by abundant light pink cytoplasm and larger nuclear size[10]; and 3) iron stores (assessment of the presence or absence of iron granules by Prussian blue iron stain, which was interpreted as normal status when iron was detectable and as deficient when iron stores were depleted).[26]

Cytologic evaluation of bone marrow involved semiquantitative determination of 1) M:E ratio: percentages of cells at each stage of myeloid and erythroid maturation, and percentages of other cells (lymphocytes, plasma cells, and macrophages) were evaluated at high power (magnification, 40×). The M:E ratio was calculated by dividing the number of all myeloid lineage cells (including mature cells) by the number of nucleated erythroid cells, 2) megakaryocyte count (number of megakaryocytes in 5 low magnification (4×) fields were counted and reported as the mean number of megakaryocytes per 5 low magnification fields, and 3) evaluation of other significant cytologic changes and presence of other cell types.

Statistical methods. Data were analyzed using commercial software (R, R Core Team, Vienna, Austria v4.0.0; R core team 2020). CBC and chemistry results were analyzed using a one-way ANOVA and paired *t* test with treatment group and time point as parameters. Bonferroni correction for multiple comparisons was performed. The mean square (variation between sample means) is reported as *M*. Variation between sample means is reported as *F*. A *P* value less than 0.05 was considered statistically significant.

Results

Groups A1 and A2 were combined and reported as a single control group. The control group showed a mild decrease in hematocrit (from an average of 43.5 to 38.5%) over the course of the study. There were no statistical differences in any of the measured parameters at baseline. All experimental groups showed an increase in hematocrit at the first measurement after dosing, followed by a gradual decrease from that value over the course of the study (Figure 1A). Hematocrit for the single dose groups was at or below baseline by week 4 after dosing, while

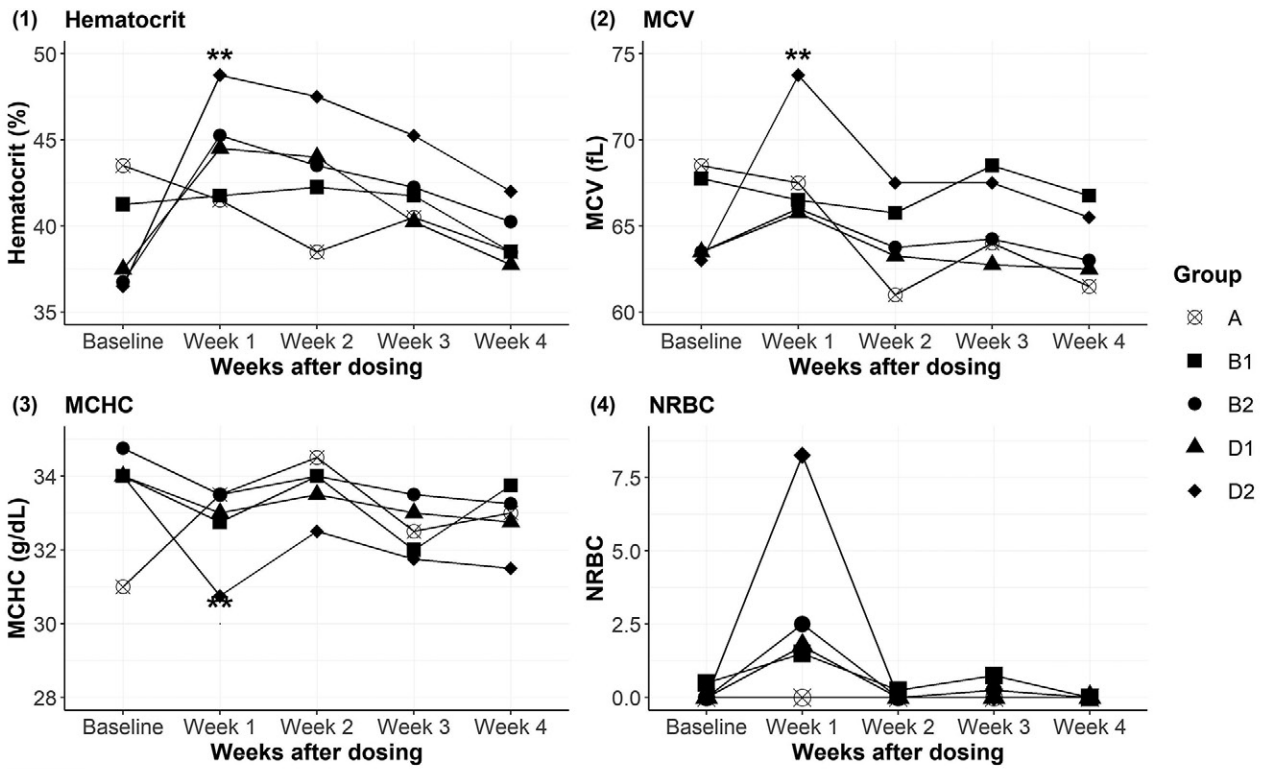

Figure 1. (A) Hematocrit mean for each dose group from baseline to week 4 after dosing. Only Group D2 had a statistically significant elevation in hematocrit (denoted by the double asterisk; ($P = 0.03$). (B) Mean corpuscular volume (MCV) mean for each dose group from baseline to week 4 after dosing. Group D2 had a significant elevation in MCV (denoted by the double asterisk) compared with all other dose groups at week 1 only ($P < 0.05$). (C) Mean cellular hemoglobin concentration (MCHC) mean for each dose group from baseline to week 4 after dosing. At week 1, group D2 showed a significant decrease in MCHC (denoted by the double asterisk; $P < 0.05$) as compared with the control and groups B2 and D1). (D) NRBCs per 100 WBC. Group D2 had a higher number of NRBCs in week 1 after dosing, but was not significantly different from numbers seen in the other groups.

hematocrit for the multidose groups remained slightly above baseline (average 36 to 41%) at week 4. The only statistically significant increase was found in group D2 at 1 week after injection [(M = 25.25), F(5, 16) = 4.78] as compared with the control group (P = 0.03) and group B1 (P = 0.008). Group D2 showed a significant elevation in mean corpuscular volume [(MCV; M = 42.27), F(5, 16) = 6.16] and a significant decrease in mean corpuscular hemoglobin concentration [(MCHC; M = 6.06), F (5, 16) = 7.78] at week 1 as compared with all dose groups except B1 for week 1 ($P < 0.05$) (Figure 1B and C).

No significant differences were seen between treatment groups at any time point for RBC, total WBC, heterophils, lymphocytes, platelets, total protein, BUN, creatinine, AST, ALT, and ALP (data not shown). The raw numbers of NRBCs was zero or one in all animals prior to EPO administration, at week 1, raw data showed 2 to 19 NRBCs in all rabbits in Group D2. However, these values were not statistically different from values in the other groups (Figure 1D). Some significant intergroup differences in chemistry parameters occurred over the course of the study. Group D1 had a significantly lower sodium than group B2 in week 3 [(M = 13.84), F(4,13) = 3.09, P = 0.039]. Group D1 had a significantly higher potassium than all other groups in week 1 [(M = 1.76), F(5,16) = 7.66, $P < 0.05$], and in week 4 had a significantly higher potassium than group B1 [(M = 0.32), F(4,13) = 3.53, P = 0.046]. In week 3, Group B1 had a significantly higher calcium compared with group D1 [(M = 2.26), F(4,13) = 14.11, P = 0.003], and group B2 had a significantly higher calcium compared with the control, group D1 and D2 [(M = 2.26), F(4,13) = 14.11, $P < 0.05$]. The cause of these differences remains unclear, and they are likely sporadic findings. Literature in various species, mainly renal failure models and human patients, shows that EPO elevates sodium,[19] potassium,[13] and intracellular calcium,[35] but our data are not consistent with those findings.

No injection site reactions were evident grossly at necropsy. Skin was not collected for histopathology because injection sites could not be identified grossly. No significant differences between the control and treatment groups were noted grossly in organs collected for histopathology. Spleen measurements and weights did not show any difference between the control and treatment groups. Three rabbits had incidental findings of ectopic splenic tissue in the pancreas that were unrelated to dose group (A2, B1, and D1).

M:E ratio was decreased in 4 rabbits (B1 = 2, D1 = 1, D2 = 1) as compared with the controls, due to expansion of the erythroid component. However, M:E ratios for all rabbits were within or close to the normal range (0.4 to 1.4),[26] except for 1 rabbit from group B2 that had a significant increase of the M:E ratio with increased myeloid component, most likely due to bronchopneumonia confirmed on histopathology in this rabbit. Bone marrow from all rabbits was normocellular, with the fat content ranging from 50% to 70% and hematopoietic cell component ranging from 30 to 50%. (Table 1). All rabbits had megakaryocyte numbers within the normal range (from 1.0 to 5.2 with a mean of 2.9 in healthy rabbits)[26] with more than 50% of the megakaryocyte lineage consisting of mature cells. Bone marrow iron stores were present in rare to moderate abundance in all rabbits without a clear difference between groups (Figure 2). All rabbits also had iron in the spleen. All livers showed congestion and mild to moderate steatosis. Other organs had no significant histopathologic findings.

Discussion

Administration of EPO raised hematocrit significantly above baseline only in rabbits that received 1,000 IU/kg in 3 consecutive doses 24 h apart (group D2). The increase in hematocrit was significant only at the first blood collection after dosing. The amount of blood drawn per week (3 mL) accounted for approximately 1% of total blood volume and would not be expected to reduce the hematocrit. Our data indicate that obtaining a week-long elevation of hematocrit and hemoglobin elevation in healthy rabbits requires repeated dosing with a high dose of EPO. A future study using anemic rabbits could determine whether this dosing regimen is effective for a longer period because neocytolysis, which is apoptosis of new erythrocytes that occurs with discontinuation of EPO administration,[16] may not occur in anemic rabbits that have lower hemoglobin and pO_2. The average life span of rabbit erythrocytes is 45 to 70 d,[5,14]

Mean corpuscular volume (MCV) is a measure of the average volume of a red blood cell. Mean corpuscular hemoglobin concentration (MCHC) is a measure of the average hemoglobin concentration of red blood cells, and directly relates to the RBC oxygen carrying capacity. In healthy humans, EPO administration increases MCV and MCHC,[16] and we anticipated a similar effect in the rabbits used in this study. A significant increase in MCV occurred in group D2 at week 1, consistent with release of larger immature erythrocytes. However, release of immature erythrocytes was not associated with a statistically significant increase in NRBCs on the blood smear of group D2 in week 1. The higher MCV occurred in association with a statistically significant reduction in MCHC; thus, cell volume increased but overall hemoglobin concentration did not. MCHC does increase in healthy humans after EPO administration, and altitude-induced hypoxia in human athletes requires over 2 wk of reduced pO_2 and subsequent endogenous EPO stimulation to produce a significant increase in hemoglobin.[17]

Histopathologic evaluation revealed minimal to no tissue changes associated with various doses of EPO. The finding of ectopic spleen in 3 rabbits is an incidental finding that has been

Table 1. Findings in postmortem bone marrow samples collected at 4 wk after initial EPO administration

		Control (n = 2)	B1 (n = 4)	B2 (n = 4)	D1 (n = 4)	D2 (n = 4)
Variable	Normal range[26]	Range (median)	Range (median)	Range (median)	Range (median)	Range (median)
Cellularity[a]	25–75%	70% (70%)	50–70% (60%)	70% (70%)	60–70% (65%)	50–60% (55%)
M:E ratio[b]	0.4–1.4	1.0–1.4 (1.2)	0.5–0.7 (0.6)	0.3–9 (4.7)[c]	0.4–1.4 (0.8)	0.5–1.2 (0.8)
Megakaryocytes[b]	1.0–5.2	5.6–7.2 (6.4)	2.6–6.4 (4.5)	5.0 (5.0)	4.0–5.0 (3.9)	1.8–4.8 (3.3)
Iron storage[a]	Detectable	Rare	Rare to moderate	Rare	Moderate	Rare to moderate

[a]Histopathology.
[b]Cytology.
[c]The broader range seen in group B2 is due to one animal with an elevated M:E ratio that had subclinical bronchopneumonia seen at necropsy and confirmed on histopathology.

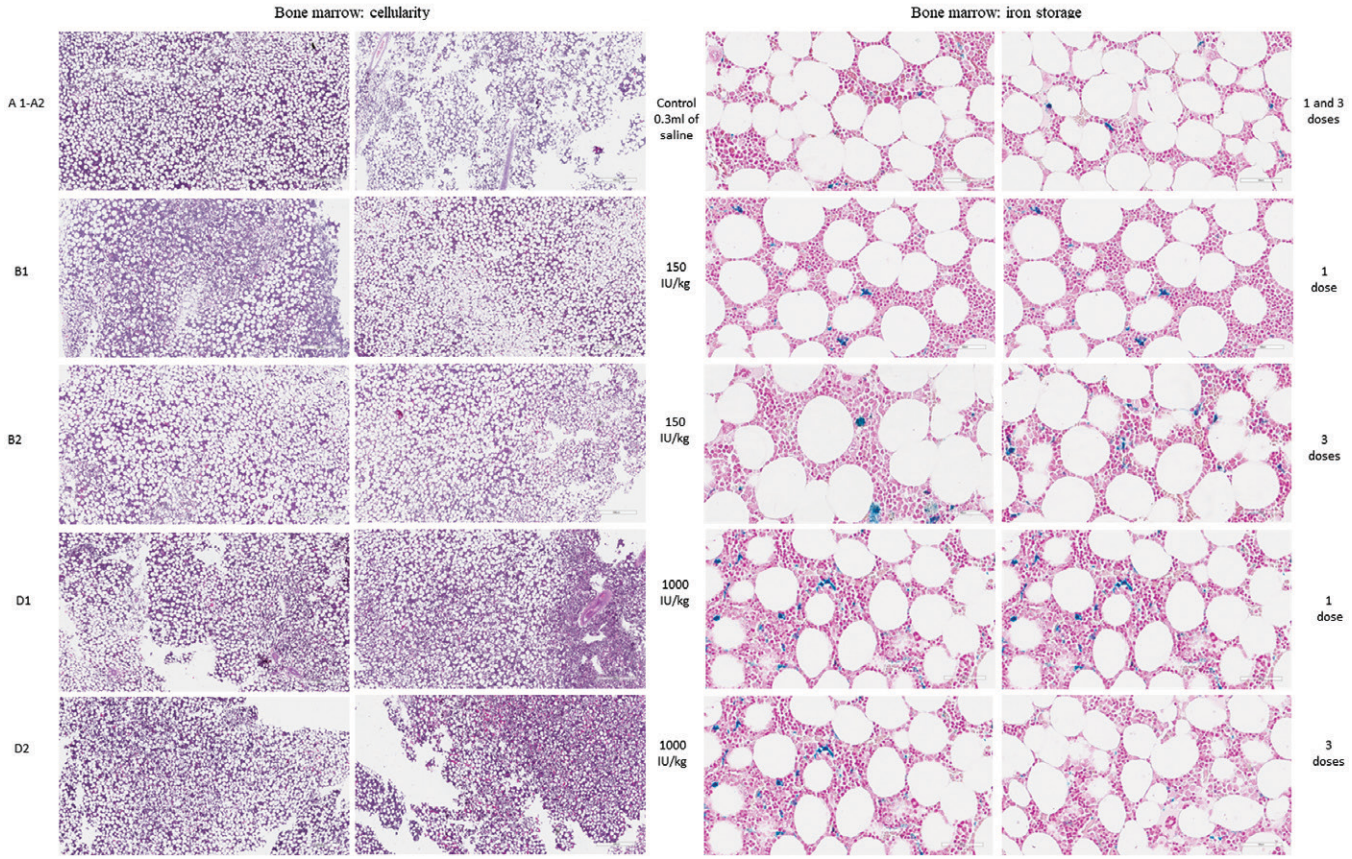

Figure 2. Bone marrow histopathology from rabbits that received erythropoietin subcutaneously. All rabbits had normal cellularity in bone marrow, with hematopoietic content ranging from 30 to 50% (H and E, 10×). All rabbits had iron in the bone marrow. The amount of iron varied from rare to moderate, with no obvious difference between the groups (Prussian Blue iron stain, 40×).

reported previously in New Zealand white rabbits.[2,31] Erythroid hyperplasia (an increased quantity of immature red blood cells) was seen in the bone marrow of 4 rabbits (2 in group B1 and one each in groups D1 and D2) but only 1 of these rabbits had a reduction of the M:E ratio that was outside the normal range. M:E ratio is calculated by comparing the number of myeloid cells to erythroid cells in bone marrow. An elevated M:E ratio may be a response to infection or inflammation or to erythroid hypoplasia (a decrease in immature red blood cells), whereas a reduced M:E ratio generally indicates erythroid hyperplasia. Because only 1 rabbit had a reduced M:E, we did not detect a dose-response relationship between the EPO dose and red blood cell expansion. Thus, based on the return to baseline by week 4 for all significant changes in CBC and chemistry parameters, any changes to hematopoietic organs were likely to be transient. Tissue changes may have been seen if collected closer to dosing. Finally, thrombolytic events are a rare complication of erythropoietin use[30]; no evidence of thrombosis was seen grossly or on histopathology.

Our data were analyzed based on treatment groups but not by sex or age because of the small number of males and the wide age range. The small size of treatment groups is a limitation of this study because sex and age are factors known to affect hematologic parameters[15,22] and may affect the baseline CBC and chemistry parameters and the bone marrow response to EPO administration. While long-term housing changes can alter rabbit hematologic parameters, these changes often occur in association with breeding[24] or changes in environmental parameters.[28] Given the absence of these variables, the brief transfer of 2 rabbits to floor pen housing for 2 wk during this study was not expected to significantly impact results. Responses associated with transportation in rabbits are characterized by hyperglycemia, heterophilia, lymphopenia, and elevations in total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK) with no significant changes in hematocrit or hemoglobin.[7] Weekly bloodwork from the 2 rabbits moved during the study did not show changes consistent with a stress response. All rabbits appeared healthy at the time of the study and had no history of significant illness, though 1 rabbit from group B2 showed evidence of bronchopneumonia at necropsy, confirmed by histopathologic evaluation. This rabbit had an increased M:E ratio, most likely due to bone marrow response to the bronchopneumonia. However, the rabbit did not display any clinical signs during the study. A second ANOVA performed without the data from this rabbit did not change to the significance of the results, so its data were included in the final analysis.

Only group D1 showed a significant short-term bone marrow response to exogenous EPO followed by a return to baseline. This group showed a significant increase in hematocrit after 1 wk. A study using longer dosage regimens could determine whether lower doses given over a longer time period would significantly increase hematocrit and hemoglobin as compared with short-term administration of higher doses. Given the cost of EPO (approximately $864 per 6 mL at the time of this study), administration of lower doses may be more cost-effective, particularly if multiple animals require prophylactic dosing.

This study showed that a dose of 150 IU/kg given for 3 d did not significantly increase hematocrit as compared with a control group. The study showed that EPO is well-tolerated

by rabbits at doses between 150 and 1,000 I/kg, as indicated by lack of changes on histopathology, including no evidence of reaction at the injection sites. However, the data indicate that short-term high doses of EPO given subcutaneously may not be an adequate prophylactic treatment for research with potential to cause anemia in rabbits. Other potential treatments for anemia in rabbits include ferrous sulfate and iron dextran.[4] Literature on these treatments in rabbits is sparse but iron administration has been shown to increase hematocrit[6] and improve histologic signs of iron deficiency in rabbits.[1] However, although generally available at a lower price than EPO, possible side effects of iron administration are more severe than those reported for EPO and include anaphylaxis. EPO and other treatments should be evaluated with regard to the efficacy, the potential risks and the financial implications in each situation. Follow-up studies evaluating more frequent low dosing of EPO over a longer period and intravenous rather than subcutaneous, dosing would be beneficial in determining the ideal dose range and route of administration for EPO in rabbits.

Acknowledgments

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. We thank Dr. Catalina Forero, Dr. Rachel Wier, Dr. Marie Brake, Dr. Rex Howard, Amir Karimi, Kaitlyn Nestor, Taylor Kirby, and the animal care technicians at the CDC Comparative Medicine Branch who provided support throughout this study.

References

1. **Abuzinadah O, Ali A.** 2008. Histologic evaluation of daily oral iron administration in anemic rabbits. Biosci Biotechnol Res Asia **5**:43–50.
2. **Adak A, Prasad MC, Lonkar PS, Kapurkar UM, Brahmankar MG, Patel MV.** 2013. Ectopic spleen in the pancreas of New Zealand white rabbits. Vet World **6**:360–362. https://doi.org/10.5455/vetworld.2013.360-362.
3. **Brown JH, Lappin TRJ, Elder GE, Bridges JM, McGeown MG.** 1990. The metabolism of erythropoietin in the normal and uraemic rabbit. Nephrol Dial Transplant **5**:855–859. https://doi.org/10.1093/ndt/5.10.855.
4. **Carpenter JW. (**2018). Exotic animal formulary, 5th edition. Amsterdam: Elsevier.
5. **Dale GL, Daniels RB.** 1991. Quantitation of immunoglobulin associated with senescent erythrocytes from the rabbit. Blood **77**:1096–1099. https://doi.org/10.1182/blood.V77.5.1096.1096.
6. **Gardner EA.** 1971. The Effects of Erythropoietin, Dibenzyline, and Iron Dextran on the Hemogram of Doe and Fetal Rabbits. (Electronic Theses and Dissertations, Document 3717), [Master of Science, South Dakota State University]. https://openprairie.sdstate.edu/etd/3717
7. **Giammarco M, Vignola G, Mazzone G, Fusaro I, Lambertini L.** 2012. Haematological parameters as indicators of transport stress in rabbits. Proceedings 10th World Rabbit Congress: Ethology, housing & welfare. 1033–1037
8. **Grasso G.** 2001. Neuroprotective effect of recombinant human erythropoietin in experimental subarachnoid hemorrhage. J Neurosurg Sci **45**:7–14.
9. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
10. **Jacobs C.** 2006. Métabolisme du fer avant et après I'ere de l'erythropoïétine. [Iron metabolism pre and post the erythropoietin era]. Nephrol Ther **2**:S313–S320. [Article in French].
11. **Jelkmann W.** 2013. Physiology and pharmacology of erythropoietin. Transfus Med Hemother **40**:302–309. https://doi.org/10.1159/000356193.
12. **Juul SE, McPherson RJ, Bauer LA, Ledbetter KJ, Gleason CA, Mayock DE.** 2008. A phase I/II trial of high-dose erythropoietin in extremely low birth weight infants: pharmacokinetics and safety. Pediatrics **122**:383–391. https://doi.org/10.1542/peds.2007-2711.
13. **Kaupke CJ, Vaziri ND.** 1993. Effect of recombinant erythropoietin on electrolytes and nutrition in end-stage renal disease patients. Int J Artif Organs **16**:59–62. https://doi.org/10.1177/039139889301600201.
14. **Kurata M, Suzuki M, Agar NS.** 1993. Antioxidant systems and erythrocyte life-span in mammals. Comp Biochem Physiol B **106**:477–487. https://doi.org/10.1016/0305-0491(93)90121-K.
15. **Laird CW, Fox RR, Mitchell BP, Blau EM, Schultz HS.** 1970. Effects of stain and age on some hematological parameters in the rabbit. Am J Physiol **218**:1613–1617. https://doi.org/10.1152/ajplegacy.1970.218.6.1613.
16. **Lundby C, Olsen NV.** 2011. Effects of recombinant human erythropoietin in normal humans. J Physiol **589**:1265–1271. https://doi.org/10.1113/jphysiol.2010.195917.
17. **Mairbäurl H.** 2018. Neocytolysis: How to get rid of the extra erythrocytes formed by stress erythropoiesis upon descent from high altitude. Front Physiol **9**:345. https://doi.org/10.3389/fphys.2018.00345.
18. **Noguchi K, Yamashiro S, Matsuzaki T, Skanashi M, Nakasone J, Miyagi K, Sakanashi M.** 2001. Effect of 1-week treatment with erythropoietin on the vascular endothelial function in anesthetized rabbits. Br J Pharmacol **133**:395–405. https://doi.org/10.1038/sj.bjp.0704083.
19. **Nushiro N, Sakamaki T, Hoshino J, Nakamura T, Sakamoto H, Imai Y, Seino M, Omata K, Sekino H, Abe K.** 1995. Recombinant human erythropoietin stimulates tubular reabsorption of sodium in anesthetized rabbits. Hypertens Res **18**:203–207. https://doi.org/10.1291/hypres.18.203.
20. **Ohls RK, Roohi M, Peceny HM, Schrader R, Bierer R.** 2012. A randomized, masked study of weekly erythropoietin dosing in preterm infants. J Pediatr **160**:790–795. https://doi.org/10.1016/j.jpeds.2011.10.026.
21. **Omlor GW, Kleinschmidt K, Gantz S, Speicher A, Guehring T, Richter W.** 2016. Increased bone formation in a rabbit long-bone defect model after single local and single systemic application of erythropoietin. Acta Orthop **87**:425–431. https://doi.org/10.1080/17453674.2016.1198200.
22. **Özcan C, Kaya A, Akgül Y.** 2012. Normal values of haematological and some biochemical parameters in serum and urine of New Zealand white rabbits. World Rabbit Sci **20**:253–259. https://doi.org/10.4995/wrs.2012.1229.
23. **Parsa CJ, Kim J, Riel RU, Pascal LS, Thompson RB, Petrofski JA, Matsumoto A, Stamler JS, Koch WJ.** 2004. Cardioprotective effects of erythropoietin in the reperfused ischemic heart. J Biol Chem **279**:20655–20662. https://doi.org/10.1074/jbc.M314099200.
24. **Pérez-Fuentes S, Muñoz-Silvestre A, Moreno-Grua E, Martínez-Paredes E, Viana D, Selva L, Villagrá A, Sanz-Tejero C, Pascual JJ, Cervera C, Corpa JM.** 2020. Effect of different housing systems (single and group penning) on the health and welfare of commercial female rabbits. Animal **14**:1270–1277. https://doi.org/10.1017/S1751731119003379.
25. **Plumbs DC.** [Internet]. Epoeitin alpha. Plumb's Veterinary Drugs. [Cited May 2020]. Available at: https://app.plumbs.com/drug-monograph/Ltw4dfSegqPROD.
26. **Riedel RM, de Matos R, Schaefer DMW.** 2017. Bone marrow cell composition and morphology in healthy juvenile female New Zealand white rabbits (*Oryctolagus cuniculus*). Am J Vet Res **78**:910–918. https://doi.org/10.2460/ajvr.78.8.910.
27. **Rölfing JHD, Bendtsen M, Jensen J, Stiehler M, Foldager CB, Hellfritzsch MB, Bünger C.** 2012. Erythropoietin augments bone formation in a rabbit posterolateral spinal fusion model. J Orthop Res **30**:1083–1088. https://doi.org/10.1002/jor.22027.
28. **Roman K, Wilk M, Ksiazek P, Czyz K, Roman A.** 2022. The effect of the housing system, season and the linseed oil ethyl esters additive on selected blood parameters in rabbits. Animals (Basel) **12**:2773–2787. https://doi.org/10.3390/ani12202773.

29. **Rosebraugh MR, Widness JA, Veng-Pedersen P.** 2012. Multidose optimization simulation of erythropoietin treatment in preterm infants. Pediatr Res **71**:332–337. https://doi.org/10.1038/pr.2011.75.
30. **Schoener B, Borger J.** 2022 Erythropoietin Stimulating Agents. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.
31. **Shao H, Peker D.** 2020. Chapter 20: Benign Hematologic Disorders Involving the Liver and Spleen, p 329–341. In: Zhang L, Shao H, Alkan S, editors. Diagnostic pathology of hematopoietic disorders of spleen and liver. Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-030-37708-3_20
32. **Shih HM, Wu CJ, Lin SL.** 2018. Physiology and pathophysiology of renal erythropoietin-producing cells. J Formos Med Assoc **117**:955–963. https://doi.org/10.1016/j.jfma.2018.03.017.
33. **Simon FHP, Erhart P, Vcelar B, Scheuerle A, Schelzig H, Oberhuber A.** 2016. Erythropoietin preconditioning improves clinical and histologic outcome in an acute spinal cord ischemia and reperfusion rabbit model. J Vasc Surg **64**:1797–1804. https://doi.org/10.1016/j.jvs.2015.10.011.
34. **Soubasi V, Kremenopoulos G, Augoustides-Savvopoulou P, Tsantali C, Kyfonidis D.** 1999. The possible role of recombinant human erythropoietin (rHuEPO) as an antioxidant in premature neonates (pilot study). Pediatr Res **45**:917. https://doi.org/10.1203/00006450-199906000-00198.
35. **Tepel M, Wischniowski H, Zidek W.** 1991. Erythropoeitin increases cytosolic free calcium concentration and thrombin induced changes in cytosolic free calcium in platelets from spontaneously hypertensive rats. Biochem Biophys Res Commun **177**:991–997. https://doi.org/10.1016/0006-291X(91)90636-L.
36. **Wang J, Wang Y, Liu K, Bi X, Sun J.** 2020. Using arterial blood as a substitute for venous blood in routine biochemistry parameter examinations in rabbits. BMC Vet Res **16**:467. https://doi.org/10.1186/s12917-020-02687-8.
37. **Xu M, Robbe VA, Jack RM, Rutledge JC.** 2010. Underfilled blood collection tubes containing K2EDTA as anticoagulant are acceptable for automated complete blood counts, white blood cell differential, and reticulocyte count. Int J Lab Hematol **32**:491–497. https://doi.org/10.1111/j.1751-553X.2009.01211.x.
38. **Yoon WH, Park SJ, Kim IC, Lee MG.** 1997. Pharmacokinetics of recombinant human erythropoietin in rabbits and 3/4 nephrectomized rats. Res Commun Mol Pathol Pharmacol **96**:227–240.