Bone Marrow Hypoplasia Induced by Administration of Estradiol Benzoate in Male Beagle Dogs

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ABSTRACT. Six male beagle dogs were administered estradiol benzoate (E₂) for 30 days at a dose of 0.1 mg/kg every day or 0.3 mg/kg every 3rd day and changes in hematology, bone marrow cells and the blood coagulation were investigated. Bleeding tendencies with severe thrombocytopenia occurred 15 or 18 days after the first E₂ injection. Three of 6 dogs died of the intra-thoracic hemorrhage on days 21, 33 and 45 respectively. White blood cells first increased to $4-10 \times 10^4/\text{mm}^3$ on days 15–21, then decreased thereafter. Red blood cells first decreased and then slowly increased after days 21–27. Platelets increased transiently soon after the E₂ administration, but then decreased rapidly and were below $3000/\text{mm}^3$ on day 15. After day 24, platelets again increased. By the bone marrow examinations, myeloid cells increased and erythroid cells decreased considerably on days 15–18. After day 24, myeloid cells decreased and erythroid cells increased and the total number of marrow cells decreased at the end of the experiment. There were no abnormal findings in the coagulation examinations in all the dogs.—**KEY WORDS:** anemia, estrogen, male dog, thrombocytopenia.

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Estrogen has been widely used in dogs as a therapeutic agent not only for genital diseases but also estrogen-sensitive neoplasia such as the circumanal gland tumor and the benign prostatic hyperplasia [3, 6]. However, it has been reported that an abnormal increase in white blood cells (WBC), a gradual decrease in red blood cells (RBC) and a severe thrombocytopenia will occur when a dog is given a large amount of estrogen [1, 2, 5, 7, 9, 14].

Although Crafts [7] and Tyslowitz [14] reported that daily administration of estradiol benzoate at a dose of 1 mg/head for 9 months did not produce any harmful side effects in dogs, recent evidence has shown that an even smaller amount of estrogen may cause bleeding episodes with severe thrombocytopenia or even death [4, 8, 11, 12].

At present, the precise etiology of these estrogen effects on the bone marrow system is still unclear, however, they may be unique to the dog because no such effects have been observed in rats, guinea pigs or rhesus mon-

keys [7, 13, 15, 16]. In human, an increased activity of coagulation factors was reported after prolonged administration of estrogen [17]. In female dogs, a sublethal dose of estrogen may cause a decrease in thrombin formation [4].

In this paper, the effects of daily administration of small doses of estradiol benzoate on the bone marrow system as well as the blood coagulation were investigated in male dogs.

MATERIALS AND METHODS

Eight 1-year-old male beagle dogs with normal findings in hematology, serum biochemical analyses and coagulation tests were divided into 3 groups. Group I (n=3) received 0.1 mg/kg of estradiol benzoate (E₂) every day, group II (n=3) received 0.3 mg/kg of E₂ every 3rd day and group III (n=2, control) were given an equivalent volume of the vehicle (sesame oil). The intramuscular injections of E₂ were given for 30 days, and

Fig. 1. Changes in RBC counts.

all the clinical observations and examinations continued for 48 days after the first injection.

Hematological examinations (RBC count, WBC count, packed cell volume, hemoglobin concentration, reticulocyte count and differential count of WBC) were performed on all the dogs every 3rd day for 30 days, and then every 6th day for the following 18 days till the experiment ended. Coagulation tests (platelet count, prothrombin time, partial thromboplastin time and plasma fibrinogen) were conducted every 3rd day for 30 days during E₂ administration, and then every 6th day for the following 18 days. Biochemical analyses (plasma total protein, serum glutamic oxaloacetic transaminase, serum alkaline phosphatase and blood urea nitrogen) were made every 6th day for 48 days. The osmotic fragility of erythrocytes was evaluated by the Coil Planet Centrifuge system (SANKI KIKAI) every 6th day during the test period.

The needle aspirations of bone marrow were carried out at the iliac crest under xylazine sedation in all the dogs every 3rd day for 30 days, and then every 6th day for the following 18 days. A differential count of 500 nucleated cells was made on the bone marrow film, and the ratio of myeloid cells to

erythroid cells (M/E ratio) was calculated.

RESULTS

Clinical observations: On day 15 or 18, all the dogs in group I and II showed bleeding tendencies such as gingival bleeding, melena and subcutaneous hematoma at the site of the bone marrow aspiration. One of the dogs in group II died of sudden respiratory distress on day 21. Autopsy of this dog revealed severe hemorrhage in the lung, endocardium, pericardium and gastrointestinal mucosa. Bleeding tendencies of the remaining dogs in this group was improved in spite of the continuous administration of E₂.

After E₂ administration ended, 2 dogs in group I died on day 33 and day 45 respectively. Their autopsies revealed the swelling of the lymph nodes, the phlegmone of the limbs and severe hemorrhages at many sites, similar to the fatality in group II. The remaining dogs of group I and II survived. The control dogs given sesame oil showed no changes in clinical observations.

Hematological findings: Red blood cell and reticulocyte counts in both group I and II decreased till day 21 and then tended to

Fig. 2. Changes in reticulocyte counts.

Fig. 3. Changes in WBC counts.

increase after day 24 although E₂ was still administered (Fig. 1 and Fig. 2).

White blood cell counts increased gradually after the start of E₂ administration, peaked at $4\text{--}10 \times 10^4/\text{mm}^3$ on day 15 or 21 (Fig. 3), and then decreased suddenly thereafter. Differential counts of blood films suggested that the increased WBC was mainly due to the increased neutrophils and their precursors,

such as myelocytes or metamyelocytes (Fig. 4). After WBC counts decreased, most neutrophils on the films were segmented. The 2 dogs in group I that died on days 33 and 45 showed severe leukopenia and their WBC counts were about $1000/\text{mm}^3$ before death. The 2 control dogs (group III) remained normal during the experiment.

Coagulation tests: The platelet counts in

Fig. 4. A peripheral blood film of a survived dog in group II on day 15. A myelocyte is observed in the center. May-Grünwald-Giemsa stain. $\times 1000$.

Fig. 5. Changes in platelet counts.

Fig. 6. Changes in prothrombin time (PT), partial thromboplastin time (PTT) and plasma fibrinogen concentrations.

Fig. 7. Changes in M/E ratios.

Fig. 8. A bone marrow film of a survived dog in group II on day 15. A remarkable increase of myeloid cells is observed. May-Grünwald-Giemsa stain. $\times 200$.

Fig. 9. A bone marrow film of a survived dog in group II on day 24. Mature myeloid cells increased and proerythroblast (arrow) reappears. May-Grünwald-Giemsa stain. $\times 400$.

Fig. 10. A bone marrow film of a dog in group I on day 36 which died on day 45. The bone marrow appears severely pancytopenic. May-Grünwald-Giemsa stain. $\times 200$.

both group I and II transiently increased after the beginning of E₂ administration, exceeded $50 \times 10^4/\text{mm}^3$ on day 3 or 6 (Fig. 5), then decreased remarkably and fell below $3000/\text{mm}^3$ on day 15. After day 24, platelet counts again showed a tendency to increase gradually, except the 2 dogs in group I which died of recurrent thrombocytopenia on days 33 and 45. Prothrombin time (PT), partial thromboplastin time (PTT) and plasma fibrinogen level did not change significantly during the experiment (Fig. 6).

Bone marrow examinations: Soon after the beginning of E₂ administration, the suppression of erythroid cell differentiation and the promotion of myeloid cell differentiation were simultaneously observed (Fig. 7). While promyelocytes and myelocytes increased in number, erythroid cells in immature form, especially the proerythroblast and the erythroblast, decreased (Fig. 8). The M/E ratio of dogs in group I and II showed peak values of 98.5–496.0 on day 15 or 18. On day 15, megakaryocytes disappeared from the mar-

row films of all the dogs receiving E₂. After day 24, the myeloid cell proliferation slowed and erythroid cell numbers tended to increase again (Fig. 9) with decreasing M/E ratios.

The 2 dogs in group I showed severe pancytopenia on their marrow films just prior to death (Fig. 10). On the marrow films of the 3 survivors, megakaryocytes appeared again on day 24, but the total number of marrow cells seemed to be less than those of control dogs.

The results of serum biochemical analyses and erythrocyte osmotic fragility in all the dogs were within the normal ranges and not significantly different between the groups.

DISCUSSION

Three of 6 dogs that received E₂ died. Early clinical symptoms after E₂ administration consisted of bleeding tendencies and gradually advancing anemia. Reduced resistance to the bacterial infection due to the severe decrease in circulating WBC was sus-

pected in the 2 mortalities of group I, which showed intra-thoracic hemorrhage associated with pneumonia, phlegmone in the limbs and swelling of lymph nodes of the whole body at autopsy.

The change in the type of the peripheral red blood cells well corresponded to the change in the erythroid proliferation in the bone marrow. Marked changes in proerythroblasts and erythroblasts during the initial stage suggest that E₂ inhibits the proliferation of erythroid cells at an early stage.

Initially, white blood cell counts increased remarkably, followed by a sudden decrease. The initial increase is presumably due to an accelerated genesis of myeloid cells, since the immature type of cells, such as promyelocytes and myelocytes abnormally increased in the bone marrow. The maximum WBC counts in peripheral blood varied among the individuals suggesting a significant difference in the tolerance to the toxic effect of E₂. Dogs with higher WBC counts at this stage have better prognosis for survival.

The bone marrow examinations revealed the suppression of erythroid cell formation and the acceleration of myeloid cell formation by E₂, which is in accordance with RBC decrease and WBC increase in the peripheral circulation. The changes in platelet counts in peripheral blood ran parallel with those of megakaryocytes on the marrow films. Initially, platelet counts increased above the normal range, and then from days 9 to 21 decreased rapidly, with the lowest value on day 21. Bleeding tendencies appeared during this period, and death occurred in a dog in group II. These changes suggest, as also reported by Perman [10], that E₂ in dogs also affects the platelet proliferation and causes bleeding episodes that could be fatal. After day 24, platelets as well as RBC and reticulocytes increased gradually. Erythroid cells and megakaryocytes appeared again in the surviving dogs even during E₂ administration. This may suggest the induction of the tolerance to

the toxic effect of E₂. The 2 dogs died after day 30 when those cells again disappeared on the bone marrow films.

PT, PTT and plasma fibrinogen were all within normal ranges, and abnormal changes in coagulation factors reported in human were not observed in this canine study. The bleeding tendencies in dogs receiving E₂ are thought simply due to thrombocytopenia, and other hemostatic disorders such as coagulation factor deficiency have been excluded.

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要 約

雄ビーグル犬におけるエストロゲン投与にともなう骨髓低形成について：三浦 昇・佐々木伸雄・小川博之¹⁾・竹内 啓（東京大学農学部家畜外科学教室，¹⁾宮崎大学農学部家畜外科学教室）——6頭の雄のビーグル犬（1才）に安息香酸エストラジオール 0.1 mg/kg の連日投与，あるいは 0.3 mg/kg の3日ごと投与を30日間行い，初回投与後48日間にわたり，臨床症状の観察，血液検査，骨髓像検査，および血液凝固検査を実施した。初回投与後15～18日に全例に重度の血小板減少をともなう出血傾向が認められ，3頭がそれぞれ21，33，45日に胸腔内出血で死亡した。白血球数は15～21日に上昇し，ついで減少傾向を示した。赤血球数は初め減少したが，21日以降は徐々に増加した。血小板数は投与開始後一旦上昇したが，その後急激な低下を示し，15日には 3000/mm³ 以下となった。生存例ではその後増加する傾向を示した。骨髓像では投与後15～18日に顆粒球系細胞が赤芽球系細胞に比較して著しく増加したが，24日以降は赤芽球系細胞が再び増加する傾向を示した。血液生化学的検査および血液凝固検査には全く異常がみられなかった。