Effect of Estradiol on Heme Biosynthetic Pathway in Lead-Poisoned Rabbits

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Abstract

Objectives: To clarify the effect of the female hormone estradiol (Est) on heme biosynthesis in lead-poisoned rabbits, parameters indicating lead exposure, such as free erythrocyte protoporphyrin (FEP) level and δ -aminolevulinic acid dehydratase (ALA-D) activity, were determined.

Methods: Twenty-six male Japanese white rabbits (body weight (BW), 3 kg) were divided into four groups: I (control), II (Est), III (Pb), IV (Est+Pb). About 3 weeks after castration, Est (3 mg/kg of BW) was injected intramuscularly, and 2 weeks thereafter, lead (1.2 mg/kg of BW) was injected intravenously. After the initial injection of each of these substances, the same dose of each of these substances was injected once a week until the 9th week.

Results: In groups III and IV, FEP level increased and ALA-D activity in the erythrocytes, bone marrow and liver decreased with an increase in lead concentration in blood. FEP level decreased significantly ($p < 0.01$) in the 8th and 10th weeks after Est injection in group IV compared to with that in group III and was not elevated in group II compared with that in group I. ALA-D activity in the erythrocytes, bone marrow and liver increased significantly in group II compared with that in group I, whereas Ht and Hb levels decreased in group II compared with those in group I, and decreased in group IV compared with those in group III. The level of iron in plasma (Fe-P) was within the normal range during experiment.

Conclusions: In this study, Est did not increase FEP level. From the above results regarding FEP level and ALA-D activity, Est may prevent an increase in FEP level caused by lead. Ht and Hb levels, which are the parameters of anemia, decreased mainly as a result of Est exposure rather than lead exposure.

Key words: estradiol, lead poisoning, free erythrocyte protoporphyrin (FEP), δ -aminolevulinic acid dehydratase (ALA-D) activity, rabbit

Introduction

Free erythrocyte protoporphyrin (FEP) level is one of the most useful indicators for evaluating chronic lead exposure. In humans and animals, the mean FEP level in females is higher than that in males (1–4). As for possible reasons for this, iron deficiency anemia (1, 5–9) and the effect of sex hormones (10–16) have been suggested, but it is not clear whether

the difference in FEP level between sexes is induced by a difference in intrinsic sensitivity between males and females, or by other factors.

Because FEP level in females starts to increase at a lower level of lead exposure than that in males (5), we have been concerned about the risk of lead exposure in female workers, even though lead work environments have been improved recently. Therefore, we have measured the FEP levels of female workers and have investigated factors affecting FEP level by carrying out human surveys and performing animal experiments (1–4).

First, in a study of healthy rural residents (1), the mean FEP level in females was higher than that in males, as also reported by Roels et al. (10). As for the parameters affecting anemia in females, Fe level in serum correlated with hematocrit

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(Ht) level, and it was suggested that women tend to have iron deficiency induced by blood loss due to menstruation, pregnancy, and differences in dietary pattern from that of males.

Second, in an experimental study of rats (Donryu) (2), a factorial analysis of variance for gain from the initial FEP level revealed that the low-lead-dose group demonstrated a significant difference between sexes ($p < 0.01$), with the female group showing an increase, but the FEP level in the group without lead injection and with low-lead-exposure was higher in males than in females.

Next, an experimental study of the effect of a female sex hormone, estradiol (Est), on FEP level was conducted in castrated rats (Donryu, male) with and without lead treatment. Est suppressed growth, decreased Ht and hemoglobin (Hb) levels, and impaired liver function, but did not show any detectable effect on FEP, urinary δ -aminolevulinic acid (ALA-U) or urinary coproporphyrin (CP-U) level. However, the erythrocyte δ -aminolevulinic acid dehydratase (ALA-D) activity in the lead plus high-dose Est group was higher than that in the lead-treatment-alone group (3).

In another experimental study (4), Japanese white (JW) rabbits, whose porphyrin metabolism is similar to that in humans, were used to clarify whether this inverse difference in FEP level between sexes compared with humans is due to a species difference, as the FEP level in rats without lead injection and with low-lead-exposure was higher in males than in females (2). The results showed that the mean FEP level in all female rabbits without lead treatment was higher than that in male rabbits, and that the FEP level in female rabbits was higher than that in male rabbits between the 1st and 3rd week after lead injection in the low-lead-dose group, and higher than that in the final week in the high-lead-dose group (t-test) (4). Furthermore, the week when FEP level began to increase in the female groups was earlier than that in the male groups in the low-lead-dose group. In the high-lead-dose group, both sexes showed an increase in FEP level from the same early time and there was no clear difference between sexes. Thus, the reason for this inverse effect of sex between rats and rabbits might be due to a difference in species or strain, and it was suggested that sensitivity to lead in females might be higher than that in males in the low-lead-dose group in both animals.

On the basis of the above results, in this study, we examined the effects of estradiol on FEP level, other parameters of lead exposure such as ALA-D activity and lead in blood (Pb-B) concentration, and parameters of anemia such as the levels of Ht, Hb and iron in plasma (Fe-P), in groups without or with lead injection using rabbits (JW, male, castrated) that show the same difference in terms of FEP level between sexes (female > male) as that in humans.

Materials and Methods

The animal experiments were performed in accordance with the Guidelines for Animal Experiments established by the Center for Animal Resources and Development, Kumamoto University.

Animals and treatment

Three replicate experiments on rabbits (BW, 3 kg; patho-

gen-free 4-month-old male JW; Biotec Co., Ltd) were carried out by the same treatment method in the same season (July–November) every year from 1999 to 2001. The rabbits ($n=9$) were castrated after preparatory feeding for 2 weeks, and after a postoperative recovery period of 2–3 weeks they were randomly divided into four groups in every experiment: group I (control, $n=2$), group II (Est, $n=2$), group III (Pb, $n=2$), group IV (Est+Pb, $n=3$). The total number of rabbits from the first to the third experiment was 26 as one rabbit of group IV in the first experiment died during the course of the experiment: group I ($n=6$), group II ($n=6$), group III ($n=6$), and group IV ($n=8$).

Feeding: Each rabbit was fed a synthetic solid diet (RM-4, Funabashi Farms) by the paired feeding method and given tap water ad libitum, in an individual cage that enabled urine collection, in a controlled room kept at $22 \pm 2^\circ\text{C}$ with relative humidities of 50–70% and a 12-hr alternating light/dark cycle during the experimental period.

Castration: After preparatory feeding for 2 weeks, all rabbits were castrated. The testes were removed (17) under anesthesia by 0.5 ml/kg of BW pentobarbital sodium injection (Nembutal 50 mg/ml, Dainippon Pharmaceutical Co., Ltd.), and ether inhalation used as required. After the removal of the testes, 5×10^4 units of benzyl penicillin potassium [0.25 ml of a solution containing 1×10^6 units benzyl penicillin K (Crystalline Penicillin G Potassium, Banyu Pharmaceutical Co., Ltd.) in 5 ml of physiologic saline for injection] was put into the cavities in the scrotum, and iodine tincture was applied to the sutured wound. For recovery, all rabbits were fed without drug treatment for 2–3 weeks after the operation.

Drug treatment: After recovery, Est (3 mg/kg of BW, administered by injecting 10 mg/ml estradiol valerate with sesame oil as an excipient; Progynon-Dpot, Nihon Schering K.K.) was administered, and 2 weeks later, lead (1.2 mg Pb/kg of BW, administered by injecting 0.732% lead acetate in a sterile 5% glucose solution; Pb 4 mg/ml) was injected once a week for 10 or 8 weeks, in the appropriate groups. Est was injected alternately in both sacrospinal muscles, and lead was injected intravenously in the ear. Sesame oil for groups I and III without Est injection, and 5% glucose solution for groups I and II without lead injection, were injected at the same volume as that used for each drug. The doses and numbers of treatments of Est and lead in each group are shown in Fig. 1.

BW was measured immediately before castration (3 weeks before the administration of Est) and every week from immediately before starting Est injection until the end of the experiment. Details are shown in Fig. 1.

Sample collection

Blood from the rabbits was collected prior to the castration (about 3 weeks before starting Est injection), immediately before starting Est injection (week 0), and at 2, 4, 6, 8 and 10 weeks after the Est injection, to determine the main parameters reflecting lead exposure and anemia.

Four milliliters of blood was drawn from ear vein, heparinized and stored cold. The levels of Ht and Hb, ALA-D activity and Pb-B concentration were measured using whole blood. The level of Fe-P, and the activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT)

Fig. 1 Average body weight (BW, kg) in each group of rabbits during experiment. There was no significant difference in BW as determined by Ryan's test for multiple comparison between any of the groups during experiment. The doses and number of treatments: Est, 3 mg/kg of BW $\times$ 10 times, injected intramuscularly in groups II and IV. Pb, 1.2 mg/kg of BW $\times$ 8 times; injected intravenously in groups III and IV. The horizontal axis: -3 wk, about 3 weeks before administration of Est (immediately before castration); 0 wk, immediately before administration of Est; and 1–10 wk, weeks after administration of Est.

were measured using plasma separated by centrifugation at 3000 rpm for 5 minutes. The levels of Ht and Hb and ALA-D activity were measured within 1 day, and the other above-stated parameters excluding Pb-B concentration were measured within 3–4 days after the blood was collected. Pb-B concentration was determined later using samples that were stored at -20°C . Separated erythrocytes were washed twice with equal volumes of 0.9% NaCl solution, resuspended in 0.9% NaCl solution at the original blood volume, stored at -20°C after measuring Ht level again, and used to determine FEP level later.

The femora and liver were removed immediately and placed on ice after exsanguination from the abdominal aorta under anesthesia induced by an intravenous injection of 0.5 ml/kg of BW pentobarbital sodium at the end of the experiment. The liver was weighed and washed in an ice-cold 0.9% NaCl solution. The excised liver and femora were wrapped in plastic film, stored cold and used for ALA-D assay within 1–2 days.

Analyses

Ht level (%) was measured using the capillary method. Hb level (g/dl) was measured using the sodium lauryl sulfate (SLS)-hemoglobin method (Hemoglobin B Test Wako, Wako Pure Chemical Industries, Ltd.). Pb-B concentration was determined by the simple standard addition method at a wavelength of 283.3 nm using a Zeeman-effect atomic absorption spectrophotometer (Model Z-9000, Hitachi Ltd.) (4). ALA-D activity in peripheral blood, bone marrow cells and the liver was measured by the method of Haas et al. (18) that was based on the Bonsignore method (19). To measure hepatic ALA-D activity, about 3 g (wet weight) of a freshly dissected liver was homogenized in 3 volumes of ice-cold 0.01 M Tris-HCl/0.9%NaCl solution with a Potter-Elvehjem homogenizer. Bone marrow cells obtained from the femora were filtered through gauze and weighed, and the entire filtrate (2–3 g wet weight) was homogenized in 3 volumes of ice-cold 0.01 M Tris-HCl/0.9% NaCl solution and the homogenate was used for ALA-D assay (20, 21). FEP level was determined fluorometrically by a partially modified method (22) of microanalysis developed by

Piomelli (23). Fe-P level was measured by the direct method using nitroso-PSAP (2-nitroso-5-[*N*-n-propyl-*N*-(3-sulfopropyl) amino] phenol) (FeC-Test Wako, Wako Pure Chemical Industries, Ltd.). The activities of GOT and GPT were measured using the Reitman-Frankel method in Karmen's unit (STA-Test Wako, Wako Pure Chemical Industries, Ltd.).

Statistical analysis

Among the four groups (three exposed subgroups and one nonexposed group) significance was tested by one-way analysis of variance (ANOVA). If the ANOVA showed significant differences ($p < 0.05$), Ryan's multiple comparison test was used to identify which subgroup or group significantly differed from the other subgroups or group.

Results

As shown in Fig. 1, there were no significant differences in mean body weight among the four groups in any week during experiment by Ryan's multiple comparison test, although some rabbits showed a loss of appetite and their BW decreased: one rabbit in group II from the 1st to the 4th week and 3 rabbits (1 died) in group IV from the 5th to the 10th week after Est injection.

As shown in Fig. 2, Ht level decreased significantly ($p < 0.01$) in group II from the 2nd to the 10th week, in group IV from the 4th to the 10th week, and in group III in the 10th week after Est injection compared with that in the control (group I). Ht level in group IV decreased from the 4th to the 10th week compared with that in group III. There were significant differences ($p < 0.01$) in the mean Ht level in the 4th and 6th weeks after Est injection between groups II and IV.

Pb-B concentrations ($\mu\text{g/dl}$ of whole blood, $\mu\text{g/dl}$ of red blood cells) increased sharply after lead injection in both lead-treated groups (III and IV) compared with that in groups I and II, which received no lead injection. As shown in Fig. 3, Pb-B concentration ($\mu\text{g/dl}$ of RBC) increased in the 4th week in group IV compared with that in group III and did not show any significant difference after that, by multiple comparison test.

Fig. 2 Average hematocrit level (Ht level, %) in each group of rabbits during experiment. The doses and number of treatments are shown in Fig. 1. Significant difference in Ht level determined by Ryan's test for multiple comparison among all the groups: a, $p < 0.05$; a^2 , $p < 0.01$ (vs group I); b, $p < 0.05$; b^2 , $p < 0.01$ (vs group II); and c^2 , $p < 0.01$ (group III vs group IV).

Fig. 3 Average lead concentration in erythrocytes (Pb-B, µg/dl of red blood cells) in each group of rabbits during experiment. The doses and number of treatments are shown in Fig. 1. Significant difference in lead concentration in erythrocytes determined by Ryan's test for multiple comparison among all the groups: a^2 , $p < 0.01$ (vs group I); b^2 , $p < 0.01$ (vs group II); and c, $p < 0.05$ (group III vs group IV).

Fig. 4 Average ALA-D activity in erythrocytes (µmoles PBG/ml of red blood cell/h) in each group of rabbits during experiment. The doses and number of treatments are shown in Fig. 1. Significant difference in ALA-D activity determined by Ryan's test for multiple comparison among all the groups: a, $p < 0.05$; a^2 , $p < 0.01$ (vs group I); and b^2 , $p < 0.01$ (vs group II).

As shown in Fig. 4, ALA-D activity in erythrocytes decreased sharply after lead injection until the final week of the experiment in the two groups treated with lead (III and IV) compared with that in the two groups without lead injection (I and II). There were no significant differences in ALA-D activity between groups III and IV, but ALA-D activity in

erythrocytes increased significantly ($p < 0.01$) in group II (Est alone) compared with that in group I from the 6th to the 10th week after Est injection.

The ALA-D activities in the bone marrow and liver in the last week of the experiment are shown in Table 1. These ALA-D activities as well as those in erythrocytes decreased in groups

Table 1 Mean ($\pm$ SD) ALA-D activities and percentages of ALA-D activity in erythrocytes, bone marrow and liver of rabbits at end of experiment

Group	N		Erythrocytes		Bone marrow		Liver	
			activity ¹⁾	%	activity ²⁾	%	activity ²⁾	%
I (Control)	6	Mean $\pm$ SD	1.835 0.215	100.0	0.263 0.051	100.0	1.688 0.293	100.0
II (Est)	6	Mean $\pm$ SD	2.123 0.337	a 115.7	0.362 0.085	a ² 137.6	2.094 0.285	a ² 124.0
III (Pb)	6	Mean $\pm$ SD	0.176 0.096	a ² , b ² 9.6	0.052 0.021	a ² , b ² 19.6	1.036 0.227	a ² , b ² 61.4
IV (Est+Pb)	8	Mean $\pm$ SD	0.204 0.092	a ² , b ² 11.1	0.070 0.024	a ² , b ² 26.6	1.211 0.183	a ² , b ² 71.8

a, $p < 0.05$; a², $p < 0.01$ (vs group I); b², $p < 0.01$ (vs group II); by Ryan's test for multiple comparison in all groups.

¹⁾, μ moles PBG/ml of RBC/h; ²⁾, μ moles PBG/g of wet weight/h.

Est, Estradiol; Pb, lead; SD, sample standard deviation; RBC, red blood cell; PBG, porphobilinogen.

Fig. 5 Average FEP level (μ g/dl of red blood cell) in each group of rabbits during experiment. The doses and number of treatments are shown in Fig. 1. Significant difference in FEP level determined by Ryan's test for multiple comparison among all the groups: a², $p < 0.01$ (vs group I); b², $p < 0.01$ (vs group II); and c², $p < 0.01$ (group III vs group IV). Groups III and IV each lost one rabbit; n=5 and 7, respectively.

III and IV compared with those in groups I and II; however, there were no significant differences in this activity between groups III and IV, whereas ALA-D activity increased in group II compared with that in group I. These findings showed that ALA-D activity in the bone marrow, liver and red blood cells is stimulated by treatment with Est alone, but no difference in this activity was found between groups III and IV, because lead strongly inhibited ALA-D activity in these two groups.

The percentages of ALA-D activity in each group compared with those of the control group in the bone marrow, liver and red blood cells in the last week of the experiment are also shown in Table 1. The percentages of ALA-D activity (%) in groups II, III and IV compared with that of group I were 116, 10 and 11 in red blood cells, 138, 20 and 27 in the bone marrow and 124, 61 and 72 in the liver, respectively. The percentages of ALA-D activity in group III (lead alone) (20% in the bone marrow and 61% in the liver) were similar to those in a previous study (Pb, 0.5 mg/kg of BW $\times$ 28 times; 3 times/week; injected intravenously) (20).

As shown in Fig. 5, FEP level increased gradually after lead injection in the two groups treated with lead (III and IV) compared with that in the two groups without lead injection (I

and II), and FEP level decreased significantly ($p < 0.01$) in the 8th and 10th weeks after Est injection in group IV compared with that in group III by Ryan's multiple comparison test. Also, there were no significant differences in FEP level between groups I and II.

As shown in Fig. 6, the mean Fe-P level in all the groups was within the normal range during experiment, but decreased significantly ($p < 0.05$) in groups II, III and IV compared with that in group I in the last week of the experiment.

GOT activity increased significantly ($p < 0.01$) in group IV compared with that in group III in the 2nd, 6th and 8th weeks after Est injection. GOT activity increased in groups II and IV compared with that in group I and decreased in group III compared with that in group II in the 8th week; however, there were no significant differences in GOT activity between groups I and III, or between groups II and IV during experiment.

GPT activity increased significantly ($p < 0.01$) in group IV compared with that in group III in the 2nd, 6th, 8th and 10th weeks after Est injection. In addition, GPT activity increased significantly ($p < 0.01$) in group IV compared with that in group I in the 6th and 8th weeks, and in group IV compared with that in group II in the 6th week.

Fig. 6 Average plasma iron level (Fe-P level, µg/dl of plasma) in each group of rabbits during experiment. The doses and number of treatments are shown in Fig. 1. Significant difference in Fe-P level determined by Ryan's test for multiple comparison among all the groups: a, $p < 0.05$; and a^2 , $p < 0.01$ (vs group I).

Discussion

We have conducted a series of studies, including this study, to investigate differences between sexes in biological parameters indicating lead exposure (mainly FEP level) in humans and animals with or without lead exposure. First, we studied the effect of Est on FEP level in castrated male rats, but found no detectable effect on FEP level (3). Therefore, in this study, we switched from rats to rabbits (JW; BW, 3 kg; male) (4), which show similar differences in FEP level between sexes to those in humans, and studied the effect of the female hormone estradiol (Est) after castration without or with lead injection, as in our previous study of rats (3).

In this study, a dose of 1.2 mg Pb/kg of BW was chosen because it is a moderate dose and results in an increase in FEP level that is not extremely high, but clearly detectable, on the basis of the findings of high-dose (Pb, 2 mg/kg of BW; 2 times/wk) and low-dose (Pb, 0.4 mg/kg of BW; 2 times/wk) treatments in our previous study of rabbits (4). We considered a dose of Est (3 mg/kg of BW) used here to be appropriate on the basis of finding of previous studies (3, 11) and the usual dose in humans of 5–10 mg per capita injected intramuscularly every 1–4 weeks.

As for the effect of Est on FEP level, which was the main issue in this study, there were no significant difference between groups I and II, that is, FEP levels was not elevated by Est alone. Furthermore, FEP level did not increase, but rather decreased significantly in the 8th and 10th weeks after Est injection in group IV (Est plus Pb) compared with that in group III (Pb) (Fig. 5). The increase in FEP level following lead administration is hardly affected by Est treatment in castrated male rats (11). Also, in this study using castrated male rabbits, FEP level was not elevated by Est, but the increase in FEP level following lead exposure was suppressed by Est. This discrepancy may be due to the differences in species, or Est may not increase FEP level.

Pb-B concentration is a parameter that increases quickly after lead exposure, and it is considered that measuring erythrocyte lead concentration (Pb-B, µg/dl of RBC) is appropriate as a means of monitoring lead exposure, as lead is mainly taken up

in red blood cells. As shown in Fig. 3, Pb-B (µg/dl of RBC) increased in group IV compared with that in group III in the 2nd week after lead injection (in the 4th week after Est injection), but this change in erythrocyte Pb-B concentration was not reflected in FEP level.

The activities of ALA synthetase (ALA-S) and ALA-D in bone marrow cells were elevated in male rats treated with estrogen, and it has been suggested that estrogen might cause erythropoietic porphyria-like symptoms (13). In our previous study of rats (3), the ALA-D activity in erythrocytes was higher in the group treated with Est plus lead than in the group treated with lead alone; however, because we did not measure ALA-D activity in the group treated with Est alone, it was not clear whether the relative increase in ALA-D activity was due to Est. Therefore, erythrocyte ALA-D activity in this study of rabbits was measured every other week. ALA-D activity in erythrocytes decreased sharply after lead injection in the 2 groups treated with lead (groups III and IV) compared with that in group I (control), and there was no significant difference in ALA-D activity between groups III and IV, but ALA-D activity in erythrocytes increased significantly in group II (Est alone) compared with that in group I from the 6th to the 10th week after Est injection as shown in Fig. 4. Also, ALA-D activity in the bone marrow and liver as well as in erythrocytes decreased in groups III and IV compared with that in group I, and even though there was no significant difference between groups III and IV, this activity increased in group II (Est alone) compared with that in group I (Table 1). Thus, the results of this study showed that ALA-D activities in the bone marrow, liver and red blood cells were increased by treatment with Est alone.

The inhibition of ALA-D by lead was also shown in the bone marrow and it suggests that ALA-D activity in peripheral blood reflected that in the bone marrow, but that heme biosynthesis in the bone marrow is performed mainly at the erythroblast stage and scarcely in peripheral blood (24). Thus, we measured ALA-D activity in the bone marrow to confirm this enzyme activity at the stage of erythropoiesis and in the liver considering the liver dysfunction caused by Est. As shown in Table 1, the results showed that the ALA-D activities of the lead-treated group decreased more sharply in peripheral blood

than in the bone marrow, and decreased slightly in the liver. These results are similar to the findings in a previous study of rabbits (Pb, 0.5 mg/kg of BW $\times$ 28 times; 3 times/week; injected intravenously) (20).

As for the reason there was no significant difference in ALA-D activity between groups III and IV in rabbits, whereas there was a significant difference in rats (3), we consider that it might have been due to a difference in the method of drug treatment or the species. First, the percentages of ALA-D activity in the erythrocytes of the lead-treated group compared with that of the control group were 15% in rats (3) and 9.6% in rabbits (Table 1); that is, ALA-D activity in rabbits is more strongly inhibited by lead than it is in rats (3). Second, in our previous paper we showed that the ALA-D activity in the lead plus high-dose-Est-treated group relative to that in the lead-treated group taken as 100% was 214% in rats (3); this suggests that Est compensated for the suppression of ALA-D activity by lead. However, the levels of ALA-D activity stimulation by Est in this study were not so high, being about 116, 138 and 124% in the erythrocytes, bone marrow and liver, respectively, compared with the control level, as shown in Table 1. From these reasons, it seemed that the difference in ALA-D activity between groups III and IV was not significant, whereas the ALA-D activities (%) in the bone marrow and liver of rabbits were 20 and 61% in group III and 27 and 72% in group IV, respectively, relative to those in group I taken as 100%, indicating a slight effect of Est.

By Est injection, growth was suppressed and Ht and Hb levels decreased in rats (3, 13, 25). Also, in this study using rabbits, Ht and Hb levels decreased in groups II (Est) and IV (Est plus Pb) compared with those in group I, and decreased consistently in group IV compared with those in group III from the 4th week after Est injection, whereas there were no significant differences in mean BW among the four groups.

As for the effect of lead on Ht and Hb levels, Ht level decreased significantly ($p<0.01$) in group III compared with that in group I in the final week of the experiment, and both parameters decreased in group IV compared with those in group II in the 4th and 6th weeks after lead administration, as shown in Fig. 2. Lead-induced anemia in humans reportedly does not occur at a Pb-B concentration less than 80 $\mu\text{g}/\text{dl}$ in many cases (26) or 100–110 $\mu\text{g}/\text{dl}$ (27), although it was reported that Hb level starts decreasing from about 50 $\mu\text{g}/\text{dl}$ Pb-B (28). The Pb-B concentrations in groups III and IV in this study were about 100 and 80–90 $\mu\text{g}/\text{dl}$, respectively. So, at this point, the extent of decrease in Hb level caused by lead-induced anemia may be poorer than that caused by Est-induced anemia.

Lead-induced anemia is due to the inhibition of heme synthesis by lead in the bone marrow. On the other hand, concerning the mechanism of estrogen-induced anemia, there is one study that showed that the bone marrow is primarily affected by estrogen resulting in estrogen-induced aplastic anemia in dogs (29), and another study that showed that Est can induce anemia through the hypoproduction of erythropoietin, a regulator of erythropoiesis, in the kidneys in rats (30).

The elevation of FEP level in iron deficiency anemia has been reported (1, 3–9, 31, 32), but in this study, the Fe-P level as a parameter of iron deficiency anemia was within the normal range in rabbits (33, 34) in all the groups during experiment, so it is seemed that FEP level does not increase as a result of iron deficiency.

Fe-P level significantly decreased in groups II, III and IV compared with that in group I in the last week of the experiment (Fig. 6), but lead was injected intravenously into the ear, and the vein had been damaged by lead by the end of the experiment, so it was impossible to administer further injections.

Estrogens including Est cause liver dysfunction. As mentioned in Result, the GOT and GPT activities, measured as indicators of liver dysfunction, in groups II (Est) and IV (Est plus lead) increased more clearly in rabbits than in rats (3), perhaps owing to Est administration. The GOT activity in a previous study of rats (3) was higher than that in these rabbits, and it has been reported that the species-dependent differences in GOT activity are as follows: humans<rabbits<rats (35).

The results of analyses of urine samples are not shown in this report. Other studies showed that ALA-U level increased in estrogen-treated rats (13), and that CP-U level increased in women taking oral contraceptives (36), and we found that the GOT and GPT activities in this study increased in the Est-treated group; therefore in a future study we should determine whether Est affects the heme biosynthetic pathway in the liver.

At the Est and lead doses used in this study, Est did not increase FEP level. Far from it, FEP level decreased in the Est plus lead treated group compared with that in the lead alone treated group. Furthermore, ALA-D activity in erythrocytes, the bone marrow and liver increased in the Est alone group, suggesting that Est compensated for the suppression of ALA-D activity by lead. From the results at this point, it might be shown that Est prevents an increase in FEP level caused by lead. Ht and Hb levels, the parameters of anemia, were decreased to a greater extent by Est treatment than by lead treatment.

The effects of other sex hormones (such as progesterone and testosterone) on the heme biosynthetic pathway remain to be clarified.

Recently, it has been well documented that several enzymes related to heme biosynthesis are contained in the Harderian glands of rodents, and differences (female>male) in porphyrin contents and in the contents of several related enzymes between sexes have been reported, and it was shown that they are controlled by hormones (3, 37–44). It may be useful to perform future studies of the Harderian glands, using animals (such as rats, mice or hamsters) smaller than rabbits.

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