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EFFECT OF PHENYLHYDRAZINE ON ANEMIC INDUCTION IN RABBITS AND SHEEP

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การศึกษาผลของ phenylhydrazine ต้องการชักนำให้กระต่าย (20 ตัว) และแกะ (5 ตัว) เป็นโรคโลหิตจางนั้น กระทำโดยการฉีด 2.5 เปอร์เซ็นต์ ของสารละลาย phenylhydrazine hydrochloride ทาง intraperitoneal ด้วยขนาด 3.0 มิลลิกรัม ต่อน้ำหนักสัตว์ทดลอง 1 กิโลกรัม การฉีด phenylhydrazine ในกระต่ายกระทำทุก 2 วัน นาน 18 วัน และในแกะฉีดทุกวัน นาน 28 วัน เมื่อครบเวลาแล้วเจาะเลือดของกระต่ายและแกะออกมา เพื่อตรวจวิเคราะห์หาระดับของฮีโมโกลบิน และฮีมาโตคริต พบว่าระดับของทั้งสองค่านี้ลดลงเฉลี่ยประมาณร้อยละ 63.2 และ 66.5 ในกระต่าย และในแกะตามลำดับ ขณะเดียวกันผู้วิจัยก็ได้ตรวจสอบหาระดับความสามารถของฮอร์โมนฮีริทโธรปอยอีติน ในพลาสมาของทั้งกระต่ายและแกะโดยวิธี radiobioassay ด้วย ผลปรากฏว่า พลาสม่าของกระต่ายมีค่าความสามารถของฮอร์โมนนี้เท่ากับ 0.19 Co Unit per mg of protein ขณะที่ค่านี้ในแกะเท่ากับ 0.17 Cobalt Unit per mg of protein.

BSTRACT

Twenty rabbits and five sheep were subjected to phenylhydrazine hydrochloride by intraperitoneal injections with dose of 3.0 mg per kilogram of body weight. The sterile solution of 2.5 per cent (w/v) phenylhydrazine hydrochloride was administered every two days for rabbits, and daily injection for sheep. It was observed that the hemoglobin concentration and hematocrit level caused by phenylhydrazine were approximately 63.2 and 66.5 per cent reduction after administrations for 18 days and 28 days in rabbits and sheep respectively. The erythropoietic activity in anemic plasma filtrate of the experimental animals was also assayed by radioisotope iron incorporation measurement. The hormonal activity of rabbits plasma was 0.19 Cobalt Unit per mg of protein while that of sheep's plasma was 0.17 Cobalt Unit per mg of protein.

INTRODUCTION

Anemia is still an important problem and commonly found in Thailand. The conditions of anemia can be artificially induced in various species of animal model by bleeding and administration of some specific drugs, or chemical agents. Primaquine, phenylhydrazine[1,2,3], isouramil and divicine[3,4,5] heve been used for this purpose during a previous time. Anemic plasma and urine of the experimental animals might become an essential source for isolation, purification and characterization of the erythropoietin or erythropoiesis stimulating hormone[6,7,8,9,10]. In this communication, the hematologic effects of phenylhydrazine on hemoglobin concentration and hematocrit level in rabbits and sheep will be investigated, and we also observed the biological activity of this particular hormone in their anemic plasma filtrate (APF) using the radioisotope iron incorporation technique.

MATERIALS AND METHODS

1. Animals :

Rabbits in both sexes weighing from 2 to 5 kilograms, male sheep with 30-40 kilograms in weight for phenylhydrazine treatment, and Albino rats in both sexes weighing from 150 to 250 g were well fed and supplied by the animal house of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

2. Chemicals :

Phenylhydrazine hydrochloride was obtained from Matheson Coleman and Bell Company, Cincinnati, U.S.A.. Sodium Chloride was taken from City Chemical Corporation, New York, U.S.A. Cobalt chloride as $CoCl_2$. 6 H_2O crystals was obtained from May and Baker Company Limited, England. Radioisotope iron (Fe-59) as ferric citrate was directly purchased from the Radiochemical Center, Amersham, U.S.A.

3. Preparation of Phenylhydrazine-Induced Anemic Plasma :

Twenty rabbits and five sheep were intraperitoneally injected with 2.5% (W/V) sterile solution of phenylhydrazine hydrochloride, with the injection dose of 3.0 mg per kilogram body weight. The injections were performed daily for sheep and every two days for rabbits. The blood of the experimental animals was frequently drawn for determination of their hemoglobin concentration and hematocrit level comparing with the normal control values. It took only 9 injections for rabbits and 28 injections for sheep. After the hemoglobin concentration and hematocrit level decreased below 10 g% and 15 volume % respectively, the experimental animals were bled by cardiac puncture. The whole blood of each animal was pooled using Acid Citrate Dextrose (ACD) solution as an anticoagulant, the plasma was collected. The anemic plasma was used for assay an erythropoietic activity.

4. Preparation of Anemic Plasma Filtrate (APF):

The preparation of APF was employed by modified method of Goldwasser (11), and of Rambach (12). The pH of anemic plasma from rabbits (500 ml), and from sheep (3.5 litres) was adjusted to be 5.5 with 0.5 M hydrochloric acid, then boiled for 10 minutes and finally filtered. The APF of each type of animals was dialyzed against deionized distilled water at 4° C for 48 hours with at least 4 changes, lyophilized and used as a "testing material" for radiobiological assay of an erythropoietic activity.

5. Determination of Hemoglobin Concentration :

Exactly 0.02 millilitre of the animal blood was pipetted using a micropipette. Five millitres of cyano-

methemoglobin of Drabkin's solution were added and mixed thoroughly. The absorbancy was measured at 540 namometer in comparison with the standard values by using Spectronic 21 Spectrophotometer.

6. Determination of Hematocrit Level :

The animal blood was drawn and placed into the balanced oxalate tube. For the microhematocrit determination method, the blood was filled into the sealed capillary tubes with approximately three-fourth of total tube volume, and then were centrifuged by using Sorvall angle-centrifuge with the speed of 4,500 rpm for five minutes. The hematocrit value in volume % was read out by a Microcapillary Reader.

7. Rodiobiological Assay of the Erythropoietic Activity :

The erythropoietic activity in "testing material" of APF was observed by the modified method of Koonanuwatchaidet (6), using the technique of radioisotope iron incorporation into red blood cells of complete starved rats. The method required at least 4 albino rats per group for testing materials and with two remaining groups for a negative control Normal Saline Solution (NSS) and a cobalt chloride standard Solution. The hormonal activity was expressed in cobalt Unit per millogram of protein of APF.

EXPERIMENTAL RESULTS

The time response curves of hemoglobin concentration and hematocrit level in rabbit and sheep to phenylhydrazine hydrochlride were illustrated in figure 1. and figure 2. respectively. From figure 1., it was clearly found that after administrations of phenylhydrazine to rabbit, the hemoglobin concentration and hematocrit rapidly lowered from the starting injection day to 8th day, and then gradually decreased to the plateau level to the last day of treatment. The hemoglobin concentration dropped from 11.8 g% to about 4.6 g%, while the hematocrit level reduced from 28 volume % to 9 volume % The average decrease of both parameters was approximately 63.2 per cent. Similarly, from figure 2., the hemoglobin concentration and hematocrit level in sheep were about 66.5 per cent lowered after complete treatments. The mean values of erythropoietic activity of rabbits and sheep were 0.19 and 0.17 Cobalt Unit per mg of protein respectively, as indicated in Table 1.

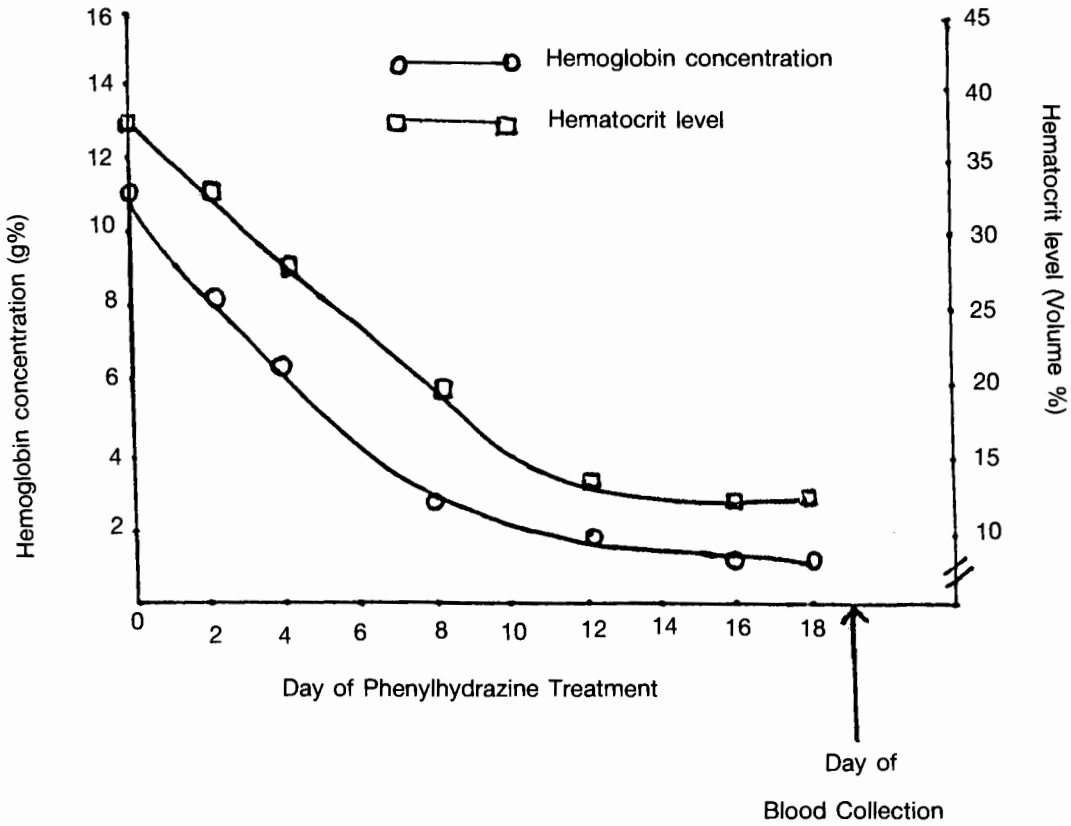

Figure 1. Effect of phenylhydrazine hydrochloride on hemoglobin concentration and hematocrit level in a rabbit.

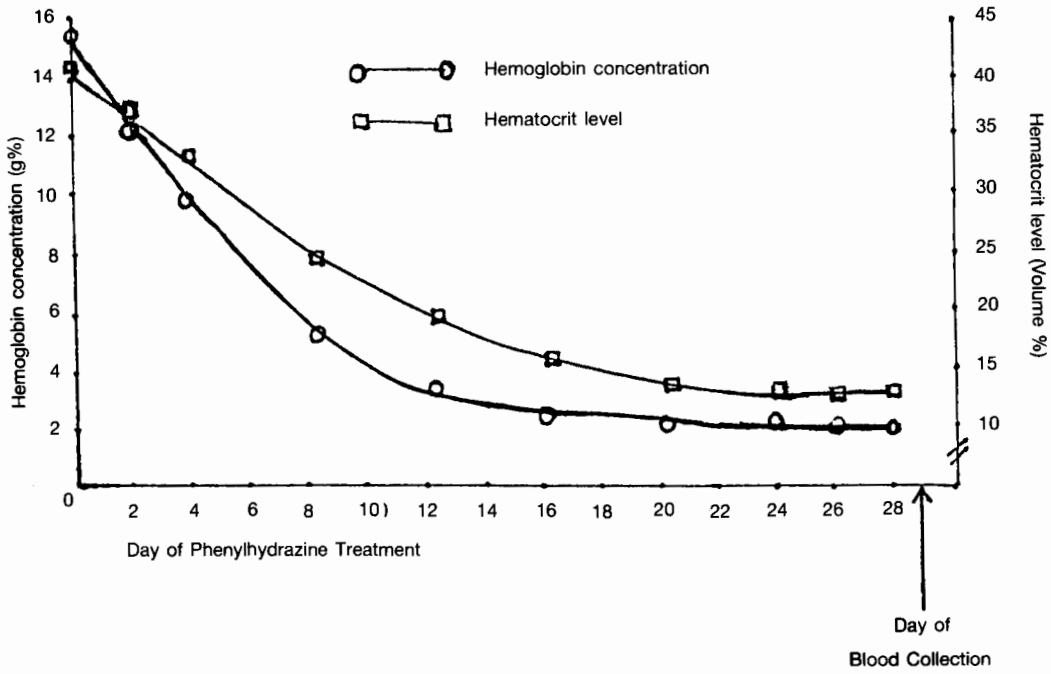

Figure 2. Effect of phenylhydrazine hydrochloride on hemoglobin concentration and hematocrit level in a sheep.

Table 1. Erythropoietic activity of APF from plasma of experimental animals induced by phenylhydrazine

Parameters	Erythropoietic Activty (Co Unit per mg of protein)	
	Rabbits	Sheep
Range	0.11-0.26	0.15-0.19
Mean	0.19	0.17

Notice : *One Cobalt Unit is equal to the erythropoietic activity by which 5 micromoles of cobalt chloride solution as a total dose injected into complete starved rat. In this paper, one cobalt unit is equivalent to 7.76 per cent of Fe-59 incorporation.*

Afer total administrations of phenyldrazine hydrochloride to the experimental animals were completed, some anemic signs were also observed, for instances; pale eyes, loss of apatite, weakness, and less activity.

DISCUSSION

In this study, it has been shown that phenylhydrazine effectively induced an anemia in both rabbits and sheep. The hemoglobin concentration and hematocrit level continuously reduced during treatments. The values markedly dropped down and became nearly constant on the 10th day and 18th day for rabbits and sheep. The plots between hemoglobin concentration and hematocrit level against the injection time likely exhibit a good correlation in both kinds of experimental animals. The difference in figures between rabbits and sheep might depend upon the blood composition; such as plasma proteins, red cell number, and total blood volume.

The anemic plasma filtrate of animals indicated the presence of the erythropoiesis stimulating hormone; or erythropoietin. It was previously reported by other investigators, phenylhydrazine was shown to secondarily affect the erythropoietin production by inhibiting the respiratory mechanism in which causing hypoxic and anemic condition(2,11,12). Moreover, it was recently demonstrated that an anemic induction by phenylhydrazine, an oxidizing agent was due to lack of enzyme activity affected red blood cell membrane damage, and iron including some other cations released from the erythrocytes (3,5,13).

The injection dose of phenylhydrazine employed in this experiment would be quite suitable. If the higher dose was treated, it probably caused sudden death to the animals. It was also observed that the withdrawn blood was easily hemolyzed, therefore, it ought to be immediately centrifuged after withdrawing

in order to avoid hemoglobin contamination and to maintain the high biological activity of erythropoietin in the plasma. However, this investigation on anemic induction in animals by using phenylhydrazine hydrochloride could be a possible technical guide for those who need to study anemia in animal models. The anemic plasma produced by animals would be perhaps useful for research on erythropoietin.

REFERENCES

1. Dern, R.J., Weinstein, I.M., LeRoy, G.V., et al. The hematologic effect of primaquine. J. Lab. Clin. Med. 1964;43:303-308.
2. Jacobson, E.M., David, A.K., and Alpen, E.L. Relative effectiveness of phenylhydrazine treatment in the production of an erythropoietic factor. Blood 1965;14:937-943.
3. Ferrali, M., Fould, I.S. and Morpurgo, L. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. Biochem. J. 1992;285: 295-301.
4. Dornfest, B.S., and Schwartz, D.A. Dexamethazone suppresses the generation of phenylhydrazine-induced anemia in the rat. Proc. Soc. Exp. Biol. Med. 1992;199:491-500.
5. Brugnara,., and Schzweda, L.I. Effect of cell age and phenylhydrazine on the cation transport properties of rabbit erythrocytes. J. Cell Physiol. 1993;154:271-280.
6. Koonnanuwatchaidet, P., and Suttajit, M. Comparative erythropoietin levels in urine of the anemic and malnourished patients. Srinagarind Med. J. 1993; 8:91-96.
7. Koury, S.T., and Bachmann, S. Erythropoietin production by the kidney. Sem. Nephrol. 1993; 13:78-86.
8. Das, R.E, Salvesen, D.R., and Camacho, J. Serum immunoreactive erythropoietin in patients with idiopathic aplastic and Fanconi's anemias. Br.J. Haematol. 1992;82:601-607.
9. Green, D., Koestner, J.A., McGraw, J.P. et al. Erythropoietin for anemia in Jahova's witness. Ann. Intern. Med. 1990;113:720-722.
10. McKenzie, D., Friedman, R., Katz, S. et al. Erythropoietin levels in anemia and kwashiokor. South Afr. Med. J. 1976; 41:1041-1048.
11. Goldwasser, E., White, W.F., and Tayer, K.B. Further purification of sheep plasma erythropoietin. Biochim. Biophys. Acta 1972;64:487-496.
12. Rambach, W.A., Alt, H.L. and Cooper, Junior, A.D. The mode of action nature of a heat-stable plasma erythropoietic factor. Blood 1967;13: 1101-1108.
13. Ferrali, M. Iron released from an erythrocyte lysate by oxidative stress is diffusible and in redox active form. FEBS Lett. 1993;15:40-44.