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Split Dose Studies on the Erythropoietic Effects of Cadmium

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The processes of red blood cell production and release, i.e., erythropoiesis, are complex and involve a series of cell divisions and maturation phases during which hemoglobin is synthesized. The formation of hemoglobin is an integral process and functions as one of the factors that control the rate and extent of erythropoiesis (Neuwirt et al. 1976). Therefore, exogenous and endogenous factors which affect the synthesis of hemoglobin would secondarily affect the rate and extent of the erythropoietic process. Incorporation of radioactively labeled iron (^{59}Fe) into the hemoglobin of erythrocyte precursor cells in the process of hemoglobin production, is a precise measurement of erythropoiesis (Gordon, 1959). Another widely used indicator of the rate and extent of hemoglobin synthesis, and thus, erythropoiesis, is the activity level of the enzyme, delta-aminolevulinic acid dehydratase (ALAD). This enzyme plays a crucial role in hemoglobin formation by promoting the condensation of two moles of aminolevulinic acid to form one mole of porphobilinogen. The degree of ALAD activity and/or its availability to perform its coupling function would, of course, affect the synthesis of hemoglobin which in turn would influence the total erythropoietic progression.

Erythropoiesis is known to be subject to a wide variety of factors including trace substances (Levander 1977). Cadmium is one such factor (Fassett 1975). This non-essential trace element has been demonstrated to induce a broad spectrum of pathophysiological conditions that are either directly or indirectly associated with erythropoiesis (Berlin and Friberg 1960, Berlin and Piscator 1961, Fox et al. 1971). The reports dealing with the relationship of cadmium and ALAD are contradictory. Cadmium has been reported to increase

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ALAD activity depending upon substrate availability (Wilson et al. 1972), to inhibit the level of ALAD's activity (Abdulla and Haeger-Aronsen 1971), and to have no observable influence on the level of ALAD activity (Roels et al. 1975). A stimulatory erythropoietic effect of cadmium as reflected by radioiron incorporation has been reported in mice (Hogan and Jackson 1986). Using other parameters and species, cadmium has been observed to cause an anemia presumably of the microcytic type (Fox et al. 1971) and to decrease the circulatory time of erythrocytes (Berlin and Friberg 1960). The purposes of the investigations presented here were to compare the erythropoietic effects of a single dosage of cadmium to split dosages. Correlations are on such effects with the activity levels of ALAD in mice treated with a single subacute treatment or with the same total dosage but separated by time.

MATERIALS AND METHODS

Female mice of the ICR strain were utilized throughout these studies. The young adult weights ranged between 25-31g with an average weight of 26.7g. Food and drinking water were freely available. Mice were randomized into control and experimental groups (N=40/group) and further divided into four subgroups composed of 10 mice each. Cadmium chloride from Pfaltz and Bauer (Stanford, CT 06902) was freshly prepared as an aqueous solution on the day of the intraperitoneal injection (day 0). Some mice received 2 mg/kg on day 0 and received no subsequent treatment while others received 1 mg/kg on day 0 and a second dosage of 1 mg/kg 12 hours later (day 1/2) or on days 1, 2, or 4. Control mice were injected with 0.2 ml of physiological saline at times comparable to the experimental animals. This volume represented the average injectate volume used for the cadmium-treated mice. A second set of controls was used; these mice received a 0.2 ml saline injection on day 0 with a 1 mg/kg body weight injection of cadmium chloride on days 1/2, 1, or 2. Another set of mice received 1 mg/kg on day 0 with no further treatment.

Animals were euthinized by cervical dislocation 24 hours after the single treatment or 24 hours after the second injection of the split dose groups. Ten control mice were sacrificed at times comparable to those of the experimental animals. Immediately after death, cardiac blood was withdrawn using sodium heparin as the anticoagulant.

A portion of the blood sample was used to ascertain packed erythrocyte volume using a micromethod (Granick et al. 1972). The enzymatic levels of ALAD of erythrocytes were determined from the whole blood sample using

modifications of the spectrophotometric micromethod as published by Granick and colleagues (1972). Enzyme activity was expressed as nmol of porphobilinogen formed/ml erythrocytes/hr. Measurement of the amount of porphobilinogen created due to the action of ALAD is an effective index of the extent of ALAD activity (Sassa et al. 1973). ALAD activity of erythrocytes from cadmium-treated mice was expressed as a percentage of the ALAD activity of erythrocytes of control mice. Differences were assigned for p values of 0.01 or less using analysis of variance.

From the blood samples the radioiron (^{59}Fe) incorporated into hemoglobin was determined from peripheral erythrocytes. Radioiron citrate/aqueous (New England Nuclear, 549 Albany St., Boston, MA 02118) was administered at a level of 0.5 micro Ci/animal. No corrections were made for ^{59}Fe activity injected/g body weight because weight variations were relatively small among animals of the experimental and control groups. A deep-well scintillation counter was used to detect radioactivity with appropriate corrections made for critical counting parameters. The ^{59}Fe uptake represents that amount utilized by hemoglobin-synthesizing erythrocytes between the time of the ^{59}Fe injection and time of sacrifice (12 hr post-^{59}Fe). The percentage values shown in the figures represent the ^{59}Fe present in erythrocytes relative to the total amount of ^{59}Fe administered. The method employed for the ^{59}Fe uptake determinations were modified from that published by Berlin and Friberg (1960). Using Analysis of Variance, statistically significant differences were assigned for p values of 0.01 or less.

RESULTS AND DISCUSSION

A dramatic elevation of ALAD activity was observed 24 hours following the 2 mg/kg body weight injection of cadmium chloride. This is shown as the striped bar of Figure I. The split dosages of 1 mg and 1 mg separated by 12 hours (stripped bar) promoted the same exaggerated increase in ALAD levels to about 82% above that of the control ($p < 0.01$). A 24-hour separation of the two treatments appeared to be effective at an approximate 60% increase above the control ALAD level. However, it is noted that splitting the 2 mg dosage by 48 and 96 hours was sufficient to prevent the elevatory effect on ALAD activity levels. The values were 23 and 9% above the control percentage, respectively, but these increases were not statistically significant ($p < 0.05$). The total dosage of 2 mg/kg body weight, whether administered as a single injection (day 0) or two 1 mg/kg injections separated by 1/2 and 1 day intervals, is an effective activator of ALAD. The single 2 mg/kg cadmium chloride

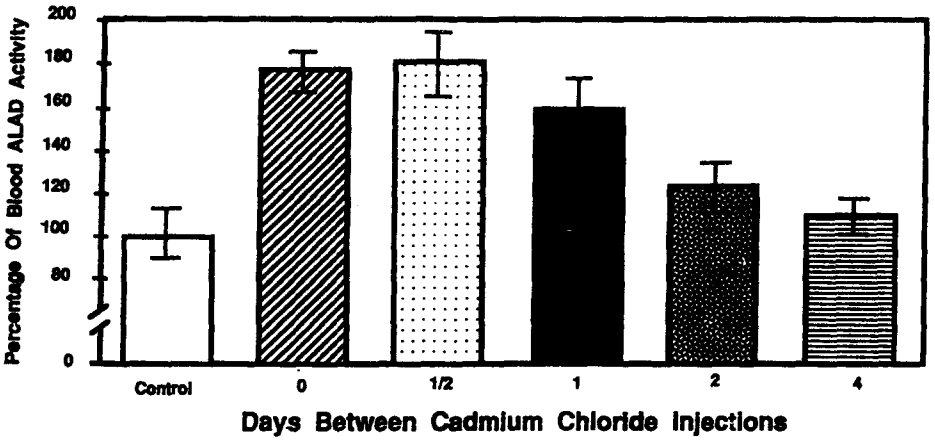

Figure 1. Blood ALAD activity of erythrocytes from mice treated with 2 mg/kg of cadmium chloride on day 0 and 1 mg/kg on days 1/2, 1, 2, or 4. Control mice were treated with saline. Vertical lines represent standard errors of the mean.

dosage has been shown to effectuate an elevation in blood ALAD activity in mice (Hogan 1987). Others have reported a similar ALAD activator effect on in vitro adult human (Davis and Avram 1978) and beef erythrocytes (Wilson et al. 1972).

Although not shown in Figure 1, a single 1 mg/kg on day 0 or 1/2, 1, 2, or 4 following a 0.2 ml injection of saline, had no effect on ALAD activity levels. The erythrocyte ALAD levels did not differ from those obtained from blood of control mice. However, separation of the 2 mg/kg total treatment split by one day did not prevent the activator effect. This would indicate that there is an additive effect of cadmium chloride on the erythrocyte target(s) for activation of ALAD. It has been shown that cadmium blocks the lead-induced suppression of ALAD activity in vitro (Davis and Avram 1978). The mechanisms of action of lead on the inhibition of the erythropoietic process has been proposed and include lead's effect on competing for hormonal responsive receptor sites of cells in the early stages of the erythropoietic progression. Thus, lead would be effective in reducing the number of cells synthesizing hemoglobin (Morse et al. 1972). Lead's action has been suggested to be direct inhibition of heme synthesis through decreasing the amount of ALAD participating in the synthesis (Kao and Forbes 1973). Cadmium's in vitro effect on the inhibitory action of lead may be due to the blocking of lead from binding with receptor sites the erythrocyte membrane and/or with the sulfhydryl groups of ALAD. In regard to the latter

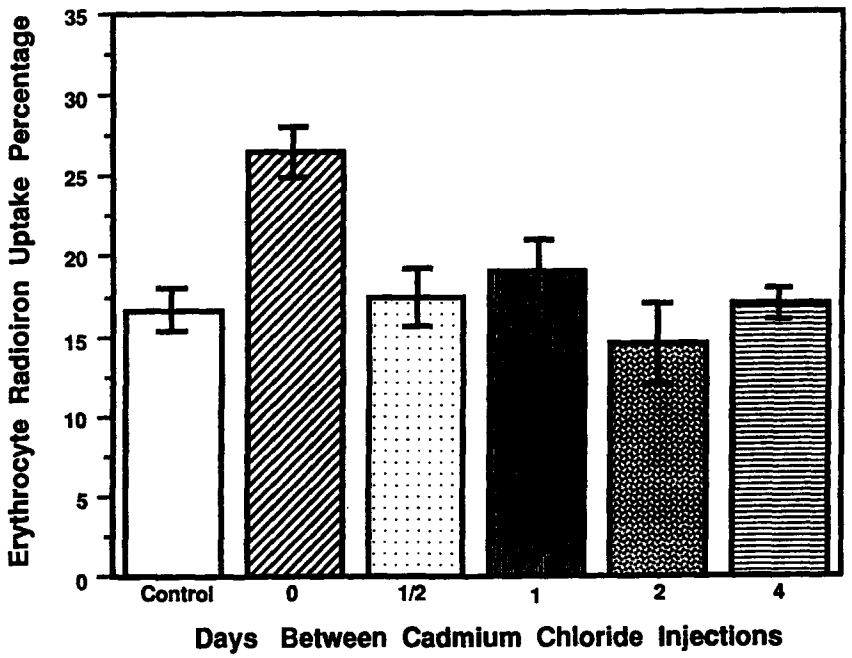

Figure 2. Percentage radioactive iron uptake of mice treated with 2 mg/kg of cadmium chloride on day 0 and with 1 mg/kg on days 1/2, 1, 2, or 4. Control mice were treated with saline. Vertical lines represent standard errors of the mean.

possibility, ALAD has been reported to contain an abundant number of sulfhydryl groups (Wilson et al. 1972). Concerning the data reported here, cadmium alone had a stimulatory effect on ALAD activation. A number of postulates could be proposed among which would include the cadmium-induced configurational changes of the enzyme through sulfhydryl group interaction. If this were the case, then the split dosage data shown in Figure 1 would suggest that a critical concentration must be available for ALAD activation and that a two-day period between dosages sufficiently lowers the cadmium titer in the blood to such a level that is subthreshold for stimulation. However, 12- and 24-hour intervals between split dosages allow the critical concentration of cadmium to be maintained and thus, promotes ALAD activation. These data may indirectly be relevant in providing some insight into the clearance time of cadmium, whereby, after the 24-hour period between the 1 mg/kg injections no subsequent ALAD effect was noted. Thus, it could be assumed that required stimulatory level was not longer present in blood, i.e., cleared from the blood between 24- and 48-hour periods.

Data in Figure 2 complement those of Figure 1. It will be noted that control ^{59}FE uptake percentage approximates 15.5. This 24-hour uptake value is consistent with values reported previously for young adult female ICR mice (Hogan and Jackson 1987). There is a time lag between stimulation of the erythropoietic system in the hemoglobin-synthesizing stages and the elevation of radioiron percentage (Fried and Gurney 1968). The time lag for the expression of ALAD inhibition, as reflected by depression radioiron uptake percentages, has been reported to be approximately 24 to 40 hours (Hogan 1990). In comparing the ALAD activity level for the 0 day group (single injection of 2mg/kg) to the corresponding ^{59}Fe uptake percentages, it will be noted that the radioiron of the 0 day group does significantly differ (p 0.01) from that of the control's value.

Recalling that blood collection occurred one day after treatment, the increase in radioiron percentage on day 0 reflects the increase in activity of ALAD as shown in Figure 1. This would suggest that the cadmium-induced activation of ALAD was protracted. The same trends were not apparent for the 1/2 and 1 day split dosage groups' ^{59}Fe uptake data, the 1/2 and 1 day groups' percentages do not significantly differ ($p<0.05$) from the control's. It appears that the second 1 mg/kg cadmium chloride treatment was necessary to effectuate an increase in radioiron into hemoglobin. This would have followed in the wake of ALAD activation after the second treatment. This notion is substantiated by the observation that a single 1 mg/kg injection of cadmium chloride had no discernible effect on either ALAD activity levels or ^{59}Fe uptake values compared to control values. The 2- and 4-day separation intervals between injections had no effect on blood ALAD activity. The radioiron data for these groups further indicate a lack of a stimulatory effect on ALAD levels. Thus, no effect was noted on increased hemoglobin synthesis as reflected by no increased uptake of ^{59}Fe.

The stimulatory effect of cadmium on the ALAD system is achieved by split dosage treatments at two sub-threshold concentrations. If the treatments are separated by more than one day, the effectiveness of the additive dosages is not detected. These data would suggest that the effect of cadmium on activation of ALAD requires a total dosage between 1 and 2 mg/kg, is relatively long-lived and is striking in the degree of stimulation for an in vivo system. Radioiron uptake percentage values further reinforce inferences concerning the mandatory total cadmium concentration administered and the latency of stimulation of the enzyme.

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