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Comparative Split Dose Effects of Selenate and Selenomethionine on Erythropoiesis of Mice

G. R. Hogan, R. E. Pendleton

College of Science and Technology, St. Cloud State University, St. Cloud, Minnesota 56301, and Veterans Affairs Medical Center, Lake City, Florida 32055, USA

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Red blood cell production and release, i.e., erythropoiesis, are complex processes involving multiple stages of cell divisions and maturation (Weiss 1977). One of the integral factors which controls erythropoiesis is the synthesis of hemoglobin. An accurate reflection of hemoglobin production is the utilization of iron into the growing heme portion of the molecule, and radioactive iron (^{59}Fe) is a commonly used tracer to measure the extend of iron uptake (Neuwirt et al. 1976). During the process of hemoglobin synthesis two moles of delta-aminolevulinic acid are coupled to form one mole of porphobilinogen. This reaction is catalyzed by the enzyme, delta-aminolevulinic acid dehydratase, ALAD (Cronkite 1973). Agents which affect the level of this enzyme and/or the mechanisms involved in iron accumulation and incorporation into hemoglobin would, of course, alter the extent and/or time course of normal erythropoiesis.

A number of reports have been published dealing with the effects of exogenous and endogenous agents which influence erythropoiesis (Levander 1977). Trace substances are among such materials. Some essential trace elements in excess or deficiency directly or indirectly affect red blood cell production and release; these include selenium. In the form of selenate, selenium has been shown to depress the incorporation of radioiron into erythrocytes (Hogan and Jackson 1986). It has also been reported that the tissue accumulation of selenium is variable according to the oxidation state of the test compound, e.g., selenate or selenite (Chen et al. 1994) or whether the selenium is an organic or inorganic form (Willhite et al. 1990, Wilber 1980).

The objectives of the studies reported here were to explore further the effects of selenium on erythropoiesis in mice by comparing the effect of an inorganic form, sodium selenate, to an organic one, selenomethionine. These compounds were administered as a total subacute dosage or as a total dosage which was separated temporally or "split" by either 12, 24, or 48 hr. This split dose technique is useful to determine the accumulated effective dosage when the total dosage is divided or

Correspondence to: G. R. Hogan

separated by various time intervals (Hogan and Raznaik 1992). If the time between dosages is sufficiently great, no effect will be noted, but, as the times are reduced, i.e., reduced times between treatments, a gradation in response may be observed. This might reflect various physiological and pharmacological parameters such as variable renal clearance rates, maximum plasma levels to create the measured effect, and an estimate of time necessary for recovery from an induced effect. ALAD activity and ^{59}Fe uptake percentages were determined at scheduled intervals following the split dosage or single treatment and analyzed in order to gain a better understanding of the dynamics of selenium and erythropoiesis.

MATERIALS AND METHODS

Young adult female ICR mice weighing between 23-29 grams were used throughout the experiments. Food and tap water were freely available at all times. Animals were randomly selected and placed in eight large groups composed of 50 mice/group. Four groups received sodium selenate while four others received selenomethionine (Aldrich Chemical Co., Inc., Milwaukee, WI and Sigma Chemical Co., St. Louis, MO, respectively). One group (N = 50) received a single intraperitoneal injection of 3 mg/kg body weight of selenium as either selenate or selenomethionine at 0 hr. Another large group received either selenate or selenomethionine at a dosage of 1.5 mg/kg at -12 hr and another 1.5 mg/kg dosage at 0 hr. Similarly, two large groups received the selenium injections as two 1.5 mg/kg treatments separated by 24 hr (-24 and 0 hr) and 48 hr (-48 and 0 hr). Mice were sacrificed by cervical dislocation at 12, 24, 48, 72, or 96 hr (10 mice/sacrifice time/selenium treatment/dosage time or times). Control mice (N = 50) received 0.2 ml sterile saline at 0 hr and were sacrificed at comparable times to those of the selenium-treated mice (10/interval). This volume represented the average injection volume of the selenium-treatments.

Immediately after euthanasia, cardiac blood was withdrawn using sodium heparin as the anticoagulant. The ^{59}Fe incorporated into hemoglobin of peripheral red blood cells was determined from the sample. Radioiron was administered 12 hours before blood harvest. The activity injected was 0.5 microCi^{59}Fe (citrate, aqueous) in a 0.1 ml volume. The time for a particular percentage of ^{59}Fe incorporation represents uptake during the 12 hr interval between injection of the radioactivity and harvest of cardiac blood. Before a sample was counted, the blood cells were rinsed twice with 4 ml chilled isotonic saline. The blood sample was then centrifuged to obtain an erythrocyte pellet. The method employed to determine the ^{59}Fe uptake has been described (Hodgson 1962). For each sample, the packed red blood cell volume was

measured by a micromethod and used for the ALAD determination using a modification of the micromethod described by Granick, et al. (1972). ALAD activity of erythrocytes obtained from control and selenate- and selenomethionine-injected mice (nmol porphobilinogen formed/ml erythrocytes/hr) was expressed as a percentage of ALAD erythrocyte activity obtained from control mice. Using Analysis of Variance, statistically significant differences were assigned for p values of 0.01 or less.

RESULTS AND DISCUSSION

Figure 1 illustrates the effects of selenate and selenomethionine treatments on blood levels of ALAD. Compared to control levels shown at 100%, at 12 hr after the single treatment (circles) or the two dosage treatment separated by 12 hr (triangles), the ALAD activity levels are approximately 20%. Separation of the dosages of selenate and selenomethionine by 24 hr (squares) showed a beneficial effect, reducing the enzyme level to only about 50 and 42%, respectively, compared to controls. The same trend is noted at 12 hr for the 48 hr-separated dosage group (diamonds), except the inhibition by selenomethionine is significantly greater ($p < 0.05$) than that from the selenate treatment. For all treatment groups recovery to control ALAD levels appears to begin between 24 and 48 hr. In the case for selenate, reestablishment of ALAD levels to the control level is progressive and complete for the 24 and 48 hr split dosage groups at the 96 hr sampling interval. However, at that time the ALAD level for the 3 mg/kg selenate treatment is significantly lower ($p < 0.01$) than the control percentage. This is the case for the 12 hr-separated split dosage group ($p < 0.05$) as well.

The selenomethionine data deviate somewhat from those of the selenate treatments. Recovery to control ALAD percentage from selenomethionine treatments appears to be slower and is not complete at 96 hr except for the 48 hr split dosage-separated group. For the other three groups, the ALAD values were significantly lower ($p < 0.01$) at 96 hr than the control value. In addition, at the sampling intervals between the 12 and 96 hr, the ALAD percentages were lower than the selenate-induced levels. These were significantly reduced ($p < 0.01$) for all samples obtained from selenomethionine-treated mice at 48, 72, and 96 hr of blood harvest and for the 24 hr-split dosage-separated group at the 24 hr harvest time.

The radioiron uptake percentages determined from selenium as selenate- and selenomethionine-treated mice, are shown in Figure 2.

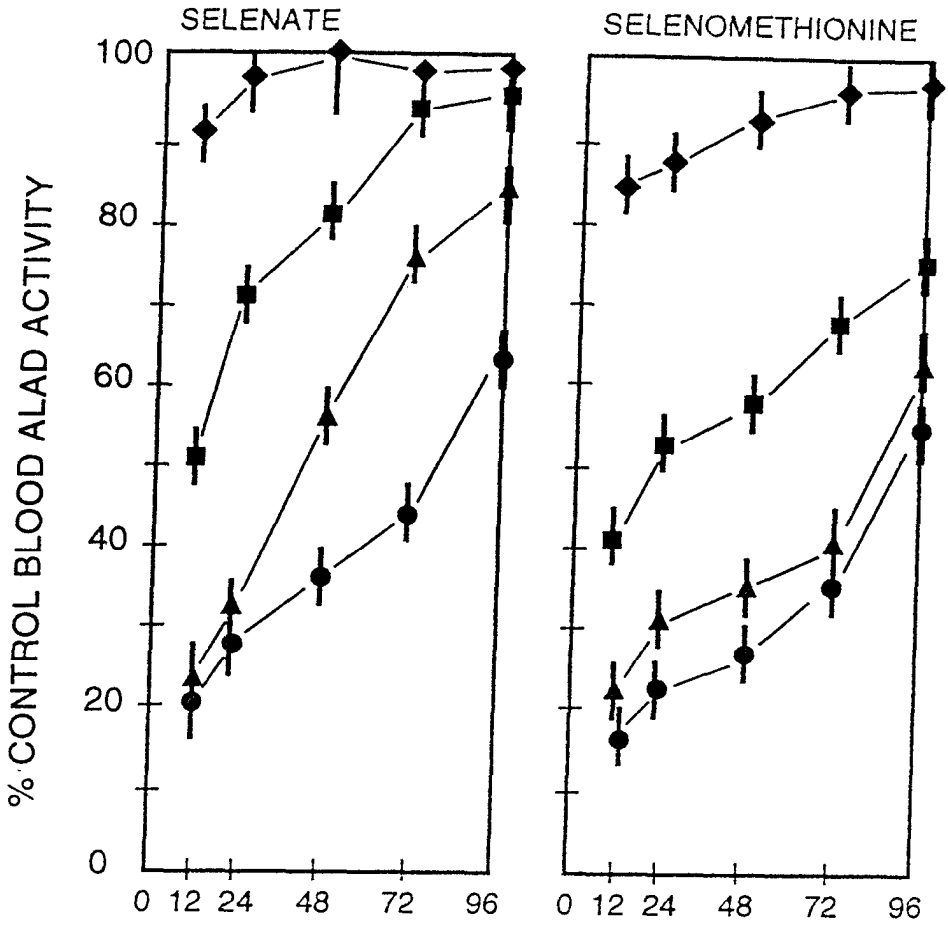

Figure 1. Blood ALAD activity of erythrocytes in mice treated with 3 mg (circles) or 1.5 mg administered at minus (-) 12 hr (triangles), -24 hr (squares) or -48 hr (diamonds). The second 1.5 mg treatment was delivered at 0 hr. ALAD levels of selenate- or selenomethionine-treated mice are compared to control values (100%). Vertical lines represent standard errors of the means.

Control percentages (open circles) ranged from approximately 18 to 20%. Both selenium treatments, however, reduced radioiron incorporation by 48 and 72 hr for the 3 mg/kg single dosage group and the 12 hr-split dosage group. In addition, the 24 hr-split dosage group receiving selenate also showed significantly lower (p less than 0.01) percentages

than controls. Maximum depression in all groups was noted 72 hr after either single or split dosage treatments. The reduced radioiron percentages of 72 hr show an increase at 96 hr. Recovery to the control percentages is more nearly achieved for the selenate groups at 96 hr; however, recovery was not complete for the single 3 mg dosage and for 12 and 24 hour-selenate split dosage treatment groups. For selenomethionine at 96 hr, recovery was complete for only the 48 hr-separated split dosage group. At that time the other three selenomethionine treatment percentages were significantly less (p less than 0.01) than comparable selenate treatment and control groups.

It has been reported that a correlation exists in regard to ALAD activity level and depressed erythropoiesis as measured by radioiron incorporation for mice receiving selenate or selenite (Hogan 1990); the results were reported to conform to a dose response relationship. Conversely, the stimulatory effect of cadmium on the erythropoietic process is directly associated with elevated ALAD levels (Hogan and Razniak 1992). In addition, it was postulated (Chisolm 1971; Kao and Forbes 1973) that the inhibition on erythrocyte production by lead administration was due to inhibition of ALAD. Early investigations proposed that a 72 hr interval is required between the onset of hemoglobin synthesis and the appearance of peripheral erythrocytes (Fried and Gurney 1968). Thus, a 72 hr interval would be necessary to detect a perturbation effect on erythropoiesis as measured by radioiron uptake in circulating red blood cells, if ALAD levels and/or activity were affected.

The data reported here support the proposed time course of an effect on decreased ALAD and the depressor response on radioiron incorporation. It is noted in Figure 1 that at 12 hr after selenate and selenomethionine at the effective dosages, ALAD levels were significantly reduced. Less hemoglobin would be produced and less radioiron would be incorporated during the interval, if ALAD activity were blocked between 0 and 12 hr post-treatment. As erythropoietic progression occurred, at 72 hr later less radioiron would be detected in the produced hemoglobin of circulating erythrocytes. This appears to be the situation as shown in Figure 2 where maximal radioiron suppression was noted at 72 hr. This is the case for both selenate and selenomethionine for the 3 mg/kg single dosage and the split 1.5 mg/kg dosages administered at 12 and 24 hr. However, in the case of the 48 hour-separated split dosage for selenomethionine, the radioiron depression is notably reduced compared to control values.

The reported data suggest that selenomethionine is a more potent agent in reducing the effectiveness and/or availability of ALAD, and

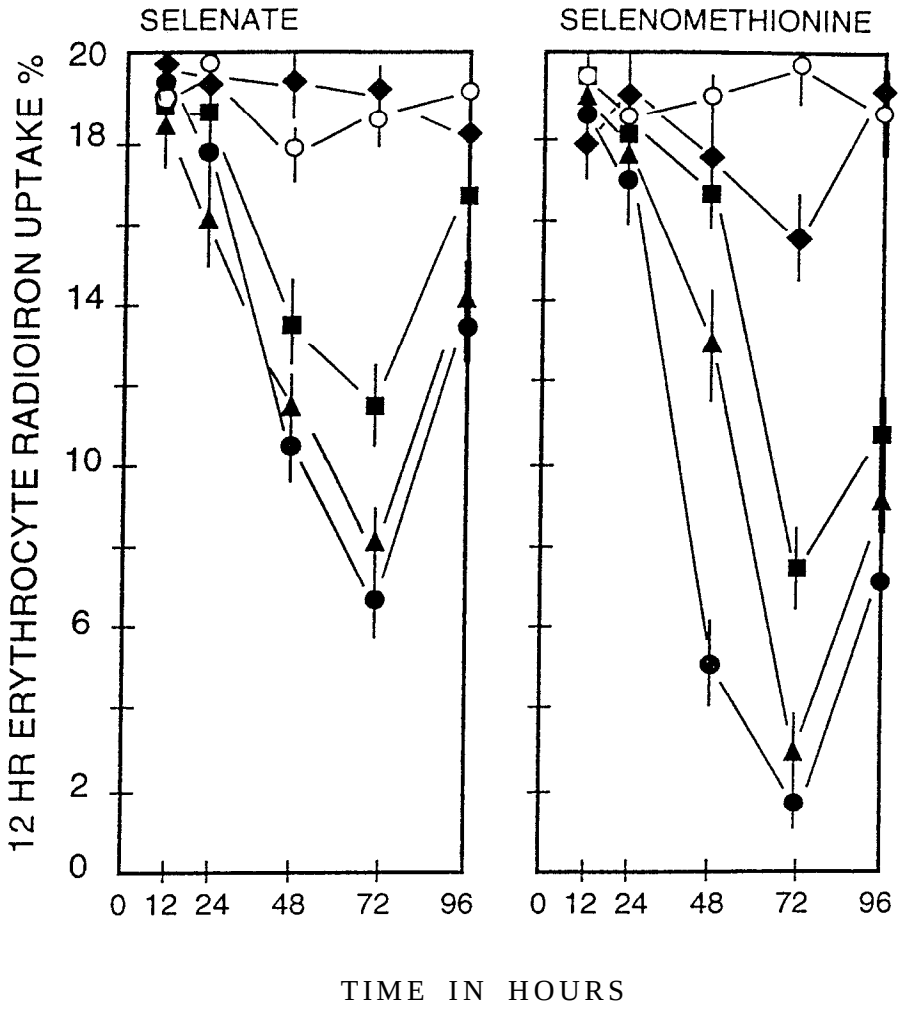

Figure 2. Percentage radioactive iron uptake of mice treated with selenate and selenomethionine at 3 mg (closed circles) or 1.5 mg administered at minus (-) 12 hr (closed triangles), -24 hr (closed squares), or -48 hr (closed diamonds) with a second 1.5 mg regimen at 0 hr. Control values are shown by open circles. Vertical lines represent standard errors of the means.

subsequently, decreasing radioiron percentage incorporation into erythrocytes. To explain the differential effectiveness of selenate and selenomethionine on erythropoietic suppression, it is tempting to postulate that these two compounds of selenium vary in the rate of tissue accumulation in erythropoietic tissues. In rodents it has been estimated that approximately 30% of erythrocyte production occurs in

the adult spleen (Hogan 1977). Chen et al., 1994 reported that in ICR mice the accumulation of selenium (Se/gm tissue) was greater and occurred earlier for selenomethionine than for sodium selenate. Their report substantiates the notion that the organic form, selenomethionine, is more rapidly retrieved from the plasma following injection into receptive tissues including spleen, and perhaps, bone marrow which is another major site of erythropoiesis. If the selenomethionine were accumulated to a greater extent and earlier, one would predict a greater depression of ALAD activity, a lesser erythropoietic progression, and thus, a reduction in radioiron incorporation of selenomethionine-treated mice compared to the selenate-treated animals. Such appears to be the case from these data.

Whatever the mechanism(s) which accounts for the differences in the selenate- and selenomethionine-induced effects as determined by these split dose studies, it is apparent that both forms of selenium exert a transient depressor effect on erythropoiesis. The effect, however, is greater for the organic form. Splitting the 3 mg/kg dosage for both selenate and selenomethionine is effective for the split dosages for each test substance separated in its administration by 12 and 24 hr. The 48-hr separation interval is markedly less effective. This may suggest that both test substances are cleared between 24 and 48 hr to reduce the impact on the ALAD system followed by a measurable reduction in ^{59}Fe incorporation. Data reported by others (Chen et al. 1994) suggest a protracted retention of renal selenomethionine compared to renal selenate which may suggest a slower clearance rate, and therefore, a longer circulatory time for the former selenium test compound.

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