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Research Article

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The Effects of Colcemid on Hematopoiesis in the Mouse

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A B S T R A C T   Colcemid was found to induce a dose and schedule dependent marrow magakaryocytosis and peripheral thrombocytosis. The response could be divided into early and late components. The early component appears to have been due to a direct stimulatory effect, probably by enhancement of endoreduplication in metaphase arrested megakaryocyte precursors. The early stimulatory response was blunted on toxic drug schedules. In contrast, the late component of the thrombopoietic response was demonstrated best on the most toxic drug schedules. It coincided temporally with the reactive restoration of the mononuclear marrow and blood cell elements, respectively. Thus, the late component appears to be a nonspecific rebound phenomenon.

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INTRODUCTION

During recent studies performed on mouse bone marrow sections after Colcemid (Ciba Pharmaceutical Company, Summit, N. J.) administration, an increase in marrow megakaryocytes was observed (1). This was of particular interest, since the increase in megakaryocytes was observed at a time when the immature nucleated cells and mature granulocytes were decreasing in number.

The chromosomal complement of morphologically recognizable megakaryocytes is tetraploid or greater (2, 3), and these cells are thought to increase their ploidy through one or more rounds of endoreduplicative DNA synthesis before cytoplasmic maturation (4–6). Presumably the earliest recognizable mega-

karyocyte arises through the endoreduplication of an unrecognizable diploid precursor. While Colcemid and related colchicine derivatives are well known for their ability to arrest cells in metaphase, they also induce endoreduplication and polyploidy in a variety of plant and animal tissues after release from metaphase block (7–10). Could it be that it is this endoreduplication promoting property of Colcemid that leads to an increase in marrow megakaryocytes?

The periwinkle alkaloids, vincristine and vinblastine, differ structurally from colchicine and its derivatives but share with them the ability to arrest cells in metaphase. Soon after the introduction of the periwinkle alkaloids into clinical medicine, a thrombocytosis was noted in some patients (11, 12). Vincristine and vinblastine have since been shown to have a therapeutic effect in thrombocytopenias of varying etiologies (13–16). Although immunosuppression (14, 17) and alterations in platelet release (18) have been suggested as possible underlying mechanisms, laboratory investigations have suggested that the thrombocytosis was secondary to an increase in marrow production, resulting from an increase in number, size, and ploidy of megakaryocytes (19–25).

The vincristine induced thrombopoietic response is dose dependent. The most pronounced thrombocytosis is observed at doses close to the minimum dose required to produce an effect. At higher doses a biphasic response is observed; the thrombocytosis is preceeded by an early thrombocytopenia (26–28).

These considerations prompted us to study the effects of Colcemid on mouse bone marrow and peripheral blood cells in relation to dosage and schedule of administration. The results of these studies are reported in this paper.

METHODS

Male National Institutes of Health all purpose white laboratory mice weighing 20–25 g were used in these experiments. Mice were given Colcemid intraperitoneally (i.p.) according to the following schecules: Group I, 2 mg/kg i.p., single injection; Group II, 14 mg/kg i.p., single injec-

tion; Group III, 2 mg/kg i.p. q.2h. × 4; Group IV, 2 mg/kg i.p. q.2h. × 7.

Peripheral blood samples from treated mice were obtained at intervals during and after Colcemid administration. Peripheral blood was collected from the subclavian artery and vein after amputation of the left forelimb of mice anesthetized with sodium pentobarbital. Platelets were counted by the phase contrast method of Brecher et al. (29). Erythrocytes and leukocytes were counted by using a model F Coulter counter. Leukocyte differentials were obtained from 100 cells in Wright-Giemsa stained smears from each animal. Reticulocyte counts were done on smears stained with brilliant cresyl blue; 1,000 erythrocytes were counted for each animal at each time point. Values for peripheral blood samples were obtained from 30 untreated control mice and from 5 mice at each experimental time point.

For the bone marrow studies, schedules I and IV were compared with untreated controls. Mice were anesthetized with sodium pentobarbital and were sacrificed by perfusion fixation at intervals as described previously (30). Five mice were sacrificed at each time point. Both femurs were removed and decalcified in 10% EDTA for seven days. 5 μm transverse histologic sections were prepared from the femur at midshaft and stained by a modification of the Feulgen technique.

In previous studies megakaryocytes were enumerated within an 80 μm wide sampling band which spanned the marrow section and passed through its center (1, 31). While this sampling method was well suited for the enumeration and spatial localization of mitoses and mononuclear cells, it was not ideal for the evaluation of megakaryocytes since the sampling band included only a fraction of the megakaryocytes present in the marrow section (5–7 megakaryocytes per sampling band as compared with 30–50 megakaryocytes per marrow section). In the present study, megakaryocytes were counted over the entire marrow section in 20–40-sections on each schedule at each time point. Histologic sections counted from the same femur were separated by at least 50 μm. The number of megakaryocytes in each section was normalized with respect to total marrow section cellular area. Since the marrow sections and central sinus were nearly circular, or approximated ellipses in shape, the cellular marrow area, A, was calculated by using the equation: $A = \pi/8 \, (D_1 D_2 - d_1 d_2)$ where D_1 and D_2 represent the longest and shortest diameter of each marrow section and d_1 and d_2 the longest and shortest diameter of the central sinus.

RESULTS

Drug toxicity. At least 70 animals were represented in each of the experimental groups. There were no deaths in the uninjected controls or in Groups II and III during the 2-wk study period. Two mice in Group I died within 36 h of injection, and one mouse in Group IV died on day 10. It is doubtful that these were drug related deaths. Mild diarrhea and reduced oral intake were noted in all groups during the first 24-h after Colcemid administration.

Colcemid effects on the marrow. In Group I (Colcemid 2 mg/kg, single injection), the number of megakaryocytes/mm² changed little during the first 8 h (Fig. 1A). A sharp rise in megakaryocytes/mm² occurred between 8 and 12 h. This increase in mega-
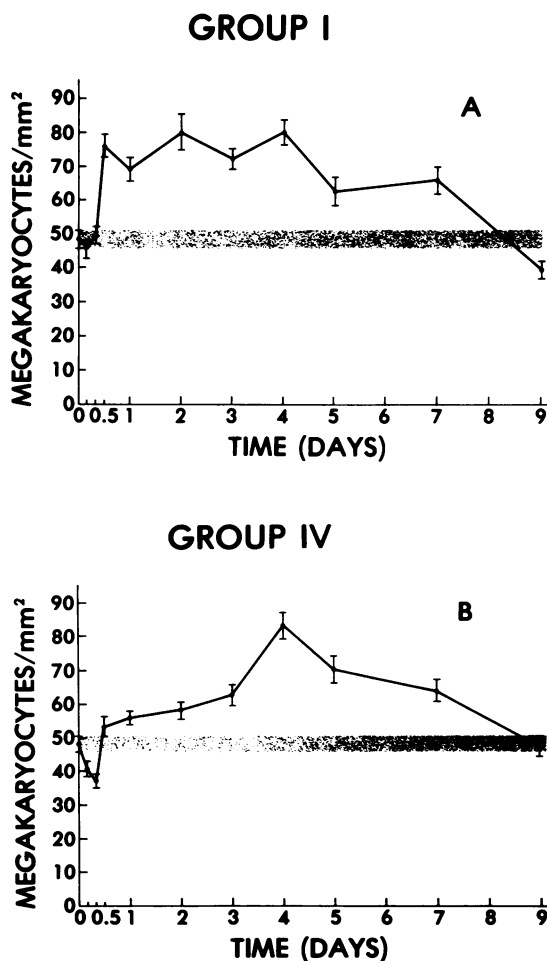

FIGURE 1 Number of megakaryocytes per mm² as a function of time after Colcemid administration. Schedule I (A) represents a single injection of Colcemid at time zero. Schedule IV (B) represents seven injections of Colcemid every 2 h over 12 h (0.5 days). Shaded bars represent control values±the standard error of the serum (SEM). Each experimental value is the average±the SEM.

karyocytes persisted through day four, (66% above control maximum) and then gradually returned to control values by day nine.

In Group IV (Colcemid 2 mg/kg, q.2h. × 7) the number of megakaryocytes/mm² fell slightly during the first 8 h (Fig. 1B), increased slightly between 8 and 24 h and then rose to a maximum of 72% above control values on day four. The megakaryocyte number then returned to control values by day nine.

On comparing the effects of Colcemid on Groups I and IV the rise in megakaryocytes can be resolved into early and late components (Figs. 1A and 1B). The early component of the rise (8–48 h) was most apparent in Group I and was blunted in Group IV. The late component (days 3 and 4) was more pronounced in Group IV.
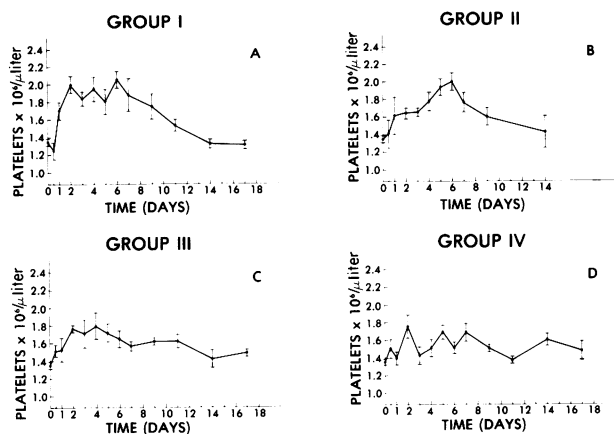
FIGURE 2 Peripheral platelet counts × 10⁶/μliter as a function of time after Colcemid administration for Groups I–IV. Shaded bars represent control values±the SEM. Each experimental value is the average±the SEM.

A fall in marrow cellularity was observed in both Groups I and IV, beginning with the 4-h time point, and reaching a nadir at 24 h. At 24 h marrow cellularity fell to 66% of control values in Group I, and 37% of controls in Group IV, as previously described (1). In Group I, total marrow cellularity returned to control values by 48 h. In Group IV total marrow cellularity was restored by day four. Thus, the early rise in megakaryocytes occurred at a time when total marrow cellularity was falling in both groups. The large rise in megakaryocytes in Group IV coincided temporally with the recovery in total marrow cellularity.

Colcemid effects on peripheral blood cells. The effects of Colcemid dosage and schedule on the peripheral platelet count are shown in Fig. 2. Thrombocytosis developed after Colcemid administration on all the schedules employed in this study. Thrombocytopenia was not observed in any of the experimental groups.

In Groups I and II an increase in peripheral platelet count was apparent between 12 and 24 h (Fig. 2A and B). The maximum increase was observed on day 6, following which platelet counts gradually decreased to control values by day 14. Maximum values of 56 and 53% above controls were quite comparable for schedules I and II, respectively, although the rise was more rapid in Group I. Peripheral platelet counts rose in Group III and IV, (Figs. 2C and D), but the respective increases of 34 and 32%, above controls were less pronounced on these multiple dose schedules than after single injections.

In contrast, peripheral leukocyte counts fell on all the Colcemid schedules employed (Fig. 3). In all groups the fall in leukocytes was apparent by 12 h, reaching a nadir on day two in Groups I and II

and on days three to four in Groups III and IV. The leukopenia was more pronounced in the multiple injection groups and most pronounced in Group IV. A rebound leukocytosis was apparent in all groups between 5 and 10 days after Colcemid administration.

There were differences between the single and multiple injection groups in the differential leukocyte patterns. In the groups receiving a single injection (I and II) the leukopenia was primarily due to a fall in lymphocytes with little change in the absolute numbers of polymorphonuclear leukocytes. In Groups III and IV the fall in polymorphonuclear leukocytes was more pronounced, and a significant rebound polymorphonuclear leukocyte leukocytosis later developed.

Colcemid's effects on the erythroid series (Fig. 4) were similar to its effects on the myeloid series. There was little change in the reticulocyte counts in Group I (Fig. 4A), whereas there was an increasing cytopenia on days one to three in Groups II, III, and IV, respectively (Figs. 4B, C, and D). A reactive reticulocytosis occurred on days four to six in Groups II, III, and IV, and was most pronounced in Group IV. In all groups there was little change in the peripheral erythrocyte count for the first five days. A rise to a maximum of 60% above control occurred on days ten to fourteen. The erythrocyte counts then declined slowly.

DISCUSSION

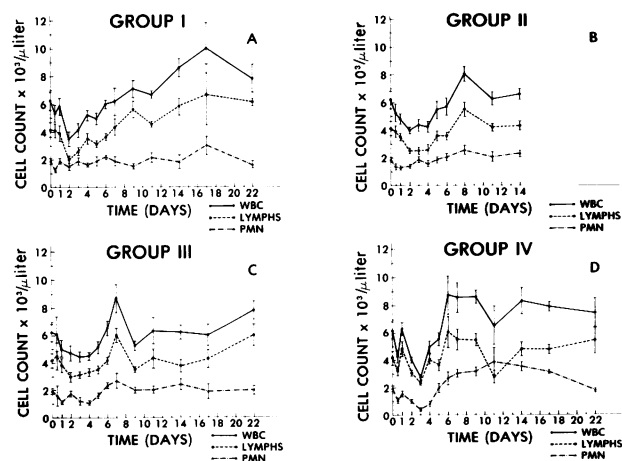In these experiments, Colcemid was found to induce a peripheral thrombocytosis the magnitude and time-

FIGURE 3 Peripheral leukocyte counts × 10³/μliter as a function of time after Colcemid administration for Groups I–IV. Leukocyte differentials of lymphocytes (Lymphs) and polymorphonuclear leukocytes (PMN) are also shown. Each experimental value is the average±the SEM. Bars represent the control values±the SEM.

course of which were dose and schedule dependent. There can be little doubt that this thrombocytosis was due in large measure to an increase in the number of megakaryocytes in the marrow. The changes in peripheral platelet count followed parallel changes in marrow megakaryocytes by 12–48 h, and the magnitudes of these changes were comparable. On schedule I, for example, a maximum increase in megakaryocytes of 66% above controls occurred on days two and four and was followed by a maximum increase of 56% in peripheral platelet count on day six.

The data suggest further that there may be two separate components to the thrombopoietic response to Colcemid. There is an early component which appears to be due to a direct stimulatory effect on the thrombopoietic maturational pathway. It consists of a sharp rise in marrow megakaryocytes at 12 h (Fig. 1A) and a corresponding rise in the peripheral platelet count at 24–48 h (Fig. 2A). The early thrombopoietic response is most pronounced on the single 2 mg/kg dose (schedule I) and appears to be blunted in the presence of drug toxicity when the dose of Colcemid is increased or duration of drug exposure is prolonged. Thus, the early rise in marrow megakaryocytes is much less pronounced on schedule IV (Fig. 1B), and the corresponding rise in peripheral platelet count is also blunted on this schedule (Fig. 2D). Blunting of the early rise in peripheral platelets is also evident on schedule II and III (Figs. 2B and 2C, respectively). It is evident from the time-course and dose response that the early thrombopoietic component is not a rebound phenomenon.

The early stimulatory effect of Colcemid is specific for megakaryocytes. The early rise in megakaryocytes occurs at a time when overall marrow cellularity is falling on both schedule I and on schedule IV. Similarly, on all drug schedules, the early rise in peripheral platelet counts at 24–48 h (Fig. 2), occurs at a time when the peripheral leukocyte counts (Fig. 3) and reticulocyte counts (Fig. 4) are falling.

In contrast, the late component appears to be a nonspecific reactive response to Colcemid's cytotoxicity and is demonstrated best on the most toxic Colcemid schedules. This component consists of a late rise in marrow megakaryocytes which is most clearly demonstrated on day three and four on schedule IV (Fig. 1B). It coincides with the reactive restoration of overall marrow cellularity and is reflected in the peripheral platelet count between days 5 and 10. The late rise in platelet count is observable on all the schedules employed but is most distinct on schedules II and IV (Figs. 2B and 2D). It coincides temporally with the reactive response observed in the circulating leukocytes (Figs. 3B–3D) and reticulocytes (Figs. 4B–4D). Other cytotoxic agents including cyclophosphamide, nitrogen mustard, cyto-
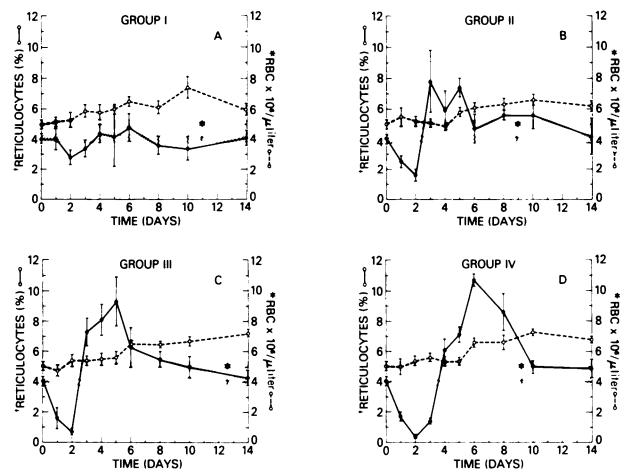

FIGURE 4   Peripheral erythrocyte (RBC) counts × $10^3/\mu$liter and reticulocyte counts as a function of time after Colcemid administration. Each experimental value is the average ±the SEM. Shaded bars represent the control values ±the SEM.

sine arabinoside, methotrexate, and 5-fluorouracil produce a similar rebound thrombocytosis (32).

The early component of Colcemid induced thrombopoiesis is of special interest. We have previously demonstrated that a single injection of 2 mg/kg of Colcemid (Group I) causes an increase in mitoses which peaks between 4 and 8 h and returns to control values by 12 h (1). On schedule IV there is a progressive accumulation of cells in metaphase during the first 8-h. The wave of mitoses falls by 12 h with a return to control value by 24 h (1). The early increase in megakaryocytes (8–12 h) develops simultaneously with the release of cells from metaphase arrest. It is clear then that the increase in megakaryocytes could not have been due to an increase in the number of divisions of unrecognized precursors. Rather, the data suggest that Colcemid increases the rate of transition from precursor to megakaryocyte. Colchicine and its derivatives are known to induce endoreduplication and polyploidy in a variety of plant and animal cell systems (7–10). Detailed studies in colchicine treated cell populations have shown that newly formed tetraploid cells are derived from cells that had previously been arrested in metaphase (7, 8). Morphologically recognizable megakaryocytes are cells with tetraploid DNA content or greater. They may achieve ploidies of 32N and 64N through repeated rounds of endoreduplicative DNA synthesis (2, 3). Presumably, the earliest recognizable megakaryocytes arise by endoreduplication from a diploid precursor line which has no distinguishing morphologic features. Spatial and temporal patterns of radioautographic labelling intensity in mouse bone marrow after tritiated thymidine administration sug-

gest that marrow megakaryocytes are derived primarily from a rapidly dividing immediate precursor pool in the subendosteal region and that the precursor-megakaryocyte transition normally occurs in cells which undergo DNA synthesis but do not divide (31). Thus, Colcemid's early thrombopoietic effect may represent the pharmacologic enhancement of a physiologic endoreduplicative process by which recognizable megakaryocytes normally arise.

The stimulation of megakaryocyte production is not restricted to colchicine and its derivations but is a property of other mitotic spindle poisons as well. The vinca alkaloids, vincristine and vinblastine, are agents whose chemical structure is unrelated to those of colchicine and its derivatives. However, both classes of agents arrest cells in mitosis as a result of binding to tubulin, a microtubular protein which is a structural component of the mitotic spindle. As with Colcemid, the vinca alkaloids produce a peripheral thrombocytosis (19–25) which has been attributed to increased platelet production (19, 20, 22, 23), secondary to an increase in marrow megakaryocytes (24), and megakaryocyte endomitosis (20). The biphasic dose response and the time-course of these changes are also similar to those observed with Colcemid (25). The most pronounced thrombocytosis is observed with doses close to the minimum dose required to produce an effect (19–25). However, at higher dosage an early thrombocytopenia is followed by a late reactive thrombopoietic response. As in the present study, this late thrombopoietic response coincides with the restoration of over-all marrow cellularity (26, 27).

It remains to be determined whether the stimulation of thrombopoiesis is a property of other mitotic spindle inhibitors (e.g., griseofulvin), agents which inhibit mitosis by other mechanisms (e.g., cytochalasin B), or other agents which delay progression through earlier stages of the cell cycle. For example, increased endoreduplication and polyploidy have been observed after exposure to Bleomycin (33) and other drugs (34, 35). These drugs may offer a variety of specific pharmacologic probes for exploring the conditions that affect the precursor–megakaryocyte transition.

Quite apart from their implications for the basic study of thrombopoiesis, the similarities and differences between Colcemid and the vinca alkaloids may have certain clinical implications as well. Vincristine and vinblastine have been found to raise the peripheral platelet count in a wide variety of clinical thrombocytopenic purpuras. Some investigators have suggested that suppression of the immune response is the mechanism underlying the thrombocytosis (17). This however, cannot explain the thrombocytosis induced in secondary thrombocytopenias or

in patients with initially normal platelet counts. It is likely that the thrombopoiesis induced by Colcemid and the vinca alkaloids shares the same underlying mechanism. The biphasic dose response observed with both classes of agents suggests that the optimal thrombopoietic doses for the vinca alkaloids may be lower than doses ordinarily employed for cytolytic cancer therapy.

Furthermore, it would appear that vincristine's therapeutic index with respect to induction of thrombocytosis may be lower than that of Colcemid. In laboratory animals the transition from maximum thrombocytosis to thrombocytopenia is observed over a twofold range of vincristine dosage (19–28). In contrast, in the present study thrombocytopenia was not observed over a sevenfold increase in Colcemid dosage.

In clinical practice the usefulness of vincristine is limited by its neurotoxicity, especially with prolonged administration. Colchicine and its derivatives lack neurotoxicity and exhibit few and relatively mild toxic effects in man even with prolonged exposure. It is possible then, that colchicine and its derivatives may be more effective and less toxic clinical thrombopoietic agents than the vinca alkaloids.

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