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Study of the Effects of Chemotherapeutic Agents on the "Early" and "Late" Responses to Erythropoietin* (32683)

GEORGE HODGSON AND IRMA ESKUCHE
Facultad de Ciencias Universidad de Chile, Santiago

The response of fasted rats to erythropoietin (EP) containing plasma, measured 24 hours after injection, the so called "early" response (1), is markedly dependant on the duration of fasting. It is maximum when EP is given at the start of fasting and drops sharply to a minimum by three days. On the other hand, the response measured 48 hours after EP the so called "late" response, is not markedly affected by the state of the recipient at the moment of injection (1). The "early" response to EP has been interpreted as reflecting the action of the hormone on erythroid precursors present at the moment of injection, and the "late" response as reflecting differentiation of erythropoietin sensitive stem cells (1).

In order to further characterize the "early" and "late" responses the effects of the following chemotherapeutic agents were studied: (a) Dactinomycin[1] known to suppress the differentiating effect of EP on stem cells in doses which do not affect other blood cells (2, 3). (b) Vinblastine[2] and methotrexate[3] (4-amino-10-methylfolic acid) drugs classified (4) as affecting cells in cycle. Vinblastine by blocking cells in mitosis (5), methotrexate by producing a thymidineless state and blocking DNA synthesis (6). A study of the effects of these drugs should given information as to whether cells involved in the "early" response must go through cycle for the response to be expressed.

Method. A × C male rats 160–180 gm were used. For the study of the early response, rats were injected intravenously at the start of fasting with 1 ml of anemic rat plasma containing 10 μ EP and a ^{59}Fe distribution assay (1) carried out 24 hours later. For the study of the "late" effect 10 μ EP were injected 2 days after the start of fasting and a ^{59}Fe distribution assay carried out 48 hours later (1). The drugs used were injected in the doses and schedules indicated in the results. Treatment with methotrexate was given in a "pulsed" form, by injecting 3 mg of leucovorin, (folinic acid), a drug factor known to reverse methotrexate effects (7), 4 hours after methotrexate injection.

Results. Methotrexate, 0.5 mg/kg, completely suppresses the early response to EP when given 4 hours after EP and reduces it to 50% and 30%, respectively, when given 4 hours before and together with EP, (Fig. 1). Only a slight effect on the "late" response is obtained when the methotrexate is given together with EP; the greatest effect is seen when it is given 4 hours before or after. The dose of methotrexate used is such that it does not affect the iron uptake of the control rats for the "early" and "late" responses.

When methotrexate, given together with EP, was not followed after 4 hours by folinic acid, the "late response observed (47 ±

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[1] Merck Research Laboratory, Rahway, New Jersey.

[2] Lederle Laboratories.

[3] Eli Lilly and Co. Indianapolis, Indiana.

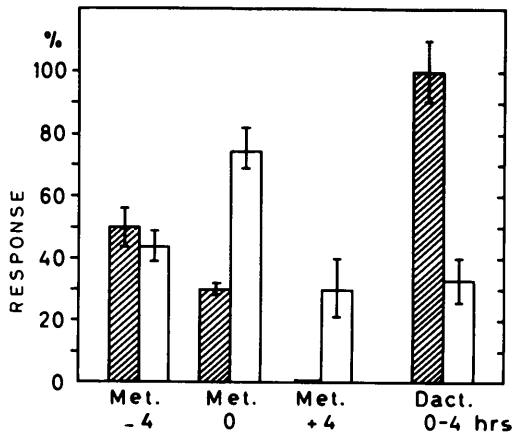

FIG. 1. Effects of methotrexate (Met.) 0.5 mg/kg and Dactinomycin 0.01 μg/gm × 4 times, 1/hr ip on the "early" and "late" response to EP. Hatched bars represent the "early" response; nonhatched, the "late" response. I = ± 1 S.E. "Early" response is that (iron uptake by erythroid tissue) measured 24 hours after EP total fast of 1 day. "Late" response is that measured 48 hours after EP total fast of 4 days.

8.0) was similar to that obtained when methotrexate was given 4 hours after EP, and followed 4 hours later by 3 mg of folinic acid. This suggests that use of folinic acid does limit the duration of ("pulses") the methotrexate effect. The effects of methotrexate "pulses" given at —9, —2, +2 hours in relation to EP injection show that sensitivity to the drug is maximum from —9 to —2 hours decreases markedly between —2, and +4 to +6 hours.

Dactinomycin in a dose 0.01 μg/gm 4 times ip, which markedly reduces the "late" response does not affect the "early" response at all (Fig. 1).

The sensitivity of the "early" and "late" responses to vinblastine (VIb) given together with EP are also different. Doses of 20 μg which nearly completely eliminate the "late" response hardly affect the "early" one (Fig. 2). When VIb is given 12 hours before EP, the effect on the "late" response is the same as when it is given togther with EP.

Discussion. The patterns of the effects of the drugs used on the "early" and "late" responses" to EP are clearly different (Fig. 1, 2).

Dactinomycin, in doses which markedly depress the "late" response to EP has no effect on the early response. This suggests that the mechanisms of the "early" and "late" responses are not the same and that an early response can occur even though the differentiating response is depressed. This would seem to rule out increase population pressure (8) due to stem cell differentiation as a mechanism for the "early" effect.

The effects of methotrexate and vinblastine on the early response would suggest, in view of their mechanism of action (4), that target cells for the "early" response to EP must go through a cell cycle for the response to manifest itself.

The difference in effects of 20 μg of vinblastine given together with EP (Fig. 2) on the "early" and "late" response, suggests, in view of the mitotic blocking action of the drug (5), that the target cell for the "early" effect goes through mitosis some time after injection of EP, at which time noneffective levels of Vlb would be present (9), when a 20 μg dose is used. On the other hand, 20 μg Vlb would affected differentiating stem cells, which

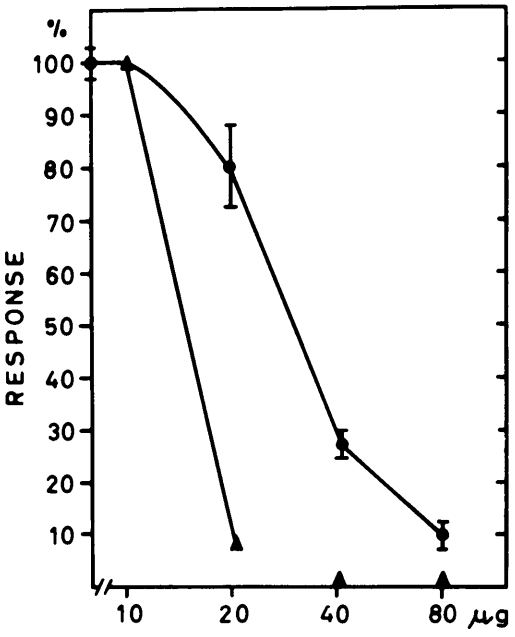

FIG. 2. Effects of different doses of vinblastine ip on the early (●) and "late" (▲) response to erythropoietin. Vinblastine was given together with EP, ± SE shown: when not indicated, it falls within the symbol.

probably go through mitosis soon after exposure to EP (10). The complete suppression of the "early" effect of EP by methotrexate given 4 hours after EP would suggest that the target cells for this effect enter into DNA synthesis phase between 4 and 8 hours after injection of EP containing plasma.

The observation that methotrexate, in the low doses used, does not completely suppress the late response, when given 4 hours after EP, could conceivably just reflect a straggling of cells, so that they are not all in S phase during the period studied. The would not be so in the case of the early response.

The data on the "early" response to EP would indicate that it is not due to stimulation of Hb synthesis by cells present at the moment of EP injection as we had suggested (1); but rather that cell proliferation is involved. The finding that methotrexate does not affect the iron uptake of the 24-hour fasted controls for the "early" effect, suggests that cell proliferation is not of marked importance in determining this uptake, as opposed to its importance in determining the increased uptake produced by EP containing anemic rat plasma. It can be assumed that the depression of erythropoiesis seen 24 hours after the start of fasting (1) reflects a depression of cell proliferation, which can be offset by the injection of anemic plasma containing EP, rather than just a decrease of stem cell differentiation. It has yet to be established whether the "early" effect is due to erythropoietin, or some other factor in the plasma. We have observed that Armour sheep plasma EP extract also shows an "early" effect, so we shall probably have to wait for a pure EP preparation before this is cleared up. Our previous observation that the early effect of EP in nonfasted animals is less than in 1 day fasted animals (1) could be a consequence of the absence of depressed cell proliferation in normal animals as opposed to the depressed proliferation produced by fasting (11).

The "late" effect in rats is very sensitive to vinblastine either given together with or 12 hours before EP. This is in agreement with previous findings, and suggests that erythropoietin responsive cells are in cycle before responding to EP and continue in cycle afterwards. If one applies Valeriote's (9) model to

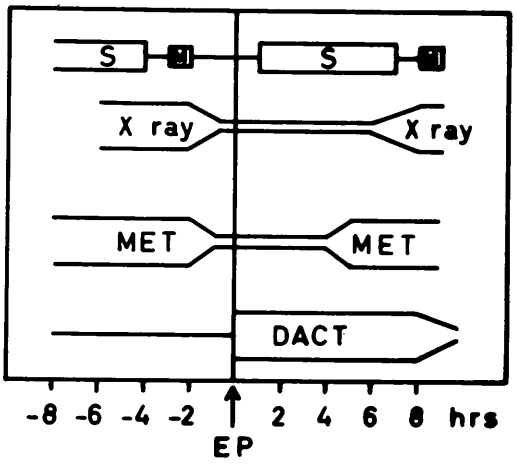

FIG. 3. Schematic represenation of the sensitivity of the "late" response to erythropoietin to X-rays, methotrexate, and Dactinomycin at different times before and after EP ejection. Also represented is the postulated mean cell cycle for an EP responsive cell. The width of the respective rectagle reprents sensitivity.

the data in Fig. 2, assuming a $T_1/_2$ of Vlb in the rat of 7–8 hours, calculated by applying criteria of biological similarity (12), and a threshold dose (DK) of 10 μg, a cycle time of about 8 hours is obtained, very similar to that of the mouse (10) and to that obtained from the accumulated mitoss curve of Vincristine treated rats (13).

The phasic changes in sensitivity to methotrexate of the late response to EP (Fig. 2) recall the changes observed in radiosensitivity (14, 15). There is a sensitive period before exposure to EP, a resistant phase afterwards, followed by another sensitive one. The second period of sensitivity to methotrexate precedes that to X-rays. These phases can be understood on the basis of the action of these agents: methotrexate blocking DNA synthesis (6), and also interferring with mitosis (7) and X-rays having a greater effect on cells in G_2, M phase (16). Figure 3 schematically represents a model of EP action adapted from that of Kretchmar (17), in which the periods of sensitivity to X-rays, methotrexate and actinomycin are shown, and a mean cell cycle for EP responsive cell is represented. Cells are assumed responsive to EP in G_1. DNA synthesis would be a prerequisite for expression of the EP effect (17). The importance of

DNA synthesis and cell division for expression of the differentiating effect of hormones has been clearly demonstrated for casein synthesis by the mammary gland (18). Sensitivity to Dactinomycin occurs (19) when cells are resistant to X-rays and methotrexate, presumably in G_1 and early S. The blocking action of actinomycin in the doses used in probably not due to interference with DNA synthesis and mitosis (20) for it has only slight effect when given at intervals up to 24 hours before EP (19), when EP responsive cells are also in cycle. Dactinomycin could conceivably act by blocking that part of the genome that must necessarily express itself for an EP responsive cell to turn into an erythroblast.

Summary. Dactinomycin in doses, 0,01 μg/gm 4 times 1/hour, which markedly depress the effect measured 48 hours after erythropoietin (EP) injection ("late" response) does not affect that measured 24 hours after EP injection ("early" response). Methotrexate 0.5 mg/kg followed 4 hours later by 3 mg of leucovorin, completely supresses the "early" response when given 4 hours after EP. The effect of methotrexate on the "late" response is phasic: Minimum when given together with, maximum when given either 4 hours before or after. Vinblastine supresses both the "early" and "late" response, although larger doses are required to affect the "early" response. The data are discussed in relation to the difference between the "early" and "late" responses and the involvement in both of cells in reproductive cycle. The "late' 'response would reflect differentiation of EP responsive cells in cycle, while the "early" response would reflect entry into cycle of erythroid cells which, in the absence of EP, would not have divided.

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