Comparison of Agents Producing a Neutrophilic Leukocytosis in Man

HYDROCORTISONE, PREDNISONE, ENDOTOXIN, AND ETIOCHOLANOLONE

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ABSTRACT To study the potential application of glucocorticosteroid administration for the measurement of the bone marrow neutrophil reserve response, blood neutrophil count changes were measured in normal subjects after the administration of intravenous hydrocortisone (25, 50, 100, 200, and 400 mg) and oral prednisone (5, 10, 20, 40, and 80 mg). The upper three doses of both steroids increased the blood neutrophil count by approximately 4,000 cells/mm^3. The neutrophilia occurring after hydrocortisone (200 mg) and/or prednisone (40 mg) was compared with that observed after endotoxin (0.8 ng/kg) and etiocholanolone (0.1 mg/kg) in 14 normal subjects, 7 patients with Wegener's granulomatosis on cyclophosphamide therapy and 10 patients with chronic idiopathic neutropenia. The normal responses (mean increase of blood neutrophils/mm^3 above base line±1 SEM) were: hydrocortisone 4,220±320, prednisone 4,610±360, endotoxin 6,060±880, and etiocholanolone 3,780±440. In the patient studies, etiocholanolone gave the smallest mean responses, but, in general, the results were similar for all agents. These data indicate that these glucocorticosteroids can be used as equivalent agents to endotoxin and etiocholanolone for measuring the neutrophil reserve response.

INTRODUCTION

Leukocytosis with an increase in blood neutrophils (PMNs)[1] is a common feature of many infectious and inflammatory diseases. This response also occurs after administration of endotoxin (1, 2), etiocholanolone (3), and glucocorticosteroids (4). The principal mechanism of the leukocytosis for each of these agents is release of PMNs from the bone marrow PMN reserves (5–7). Since a ready reserve of PMNs is regarded as a critical element of normal host defenses against infection, marrow reserve responses have been used to estimate the amount of myelotoxic chemotherapy that will be tolerated by patients with inflammatory and neoplastic diseases (8, 9). In addition, marrow reserve responses have been measured in various diseases associated with neutropenia to further basic understanding of the pathophysiology of these conditions (10–12).

Most investigators have used endotoxin or etiocholanolone to study the marrow reserves (8–15). Neither of these agents are widely available, and both cause fever, pain, and other significant symptoms (1–3). The few studies in which corticosteroids have been used to measure the capacity to develop a neutrophilic leukocytosis suggest that the response to corticosteroids may parallel the response to etiocholanolone and endotoxin (4, 10, 16–20). Since single dose administration of hydrocortisone or prednisone is generally not accompanied by side effects and since these steroids are readily available, it would be advantageous if they were used as the basic marrow reserve test agents. We therefore have determined in normal subjects the dose-response relationships for the neutrophilia produced by oral prednisone and intravenous hydrocortisone and compared the PMN responses to these agents with the responses to endotoxin and etiocholanolone. To evaluate the clinical applicability of hydrocortisone for testing the marrow granulocyte reserves, we have compared the PMN response to hydrocortisone with the

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[1] *Abbreviation used in this paper:* PMN, blood neutrophil.

response to etiocholanolone and endotoxin in patients with Wegener's granulomatosis on cyclophosphamide therapy and patients with chronic idiopathic neutropenia.

METHODS

Subjects. All studies were performed after obtaining informed consent from the patients and volunteers. None of the patients were receiving corticosteroids and none of the volunteers were receiving any medications. During all studies, the subjects were hospitalized and restricted to bed-chair activity. Meals were not withheld.

Dose-response studies. 11 normal adult volunteers of either sex, ages 20–27 yr, received single intravenous doses of 25, 50, 100, 200, and 400 mg of hydrocortisone sodium succinate (The Upjohn Company, Kalamazoo, Mich.) at 8:00 a.m. on separate days at least 2 days apart. A second group of 15 volunteers received single doses of 5, 10, 20, 40, and 80 mg of prednisone (The Upjohn Company) at 8:00 a.m. on separate days at least 2 days apart. A third group of nine subjects received no drug. In all three groups, PMN counts were made before and hourly for 6 h after beginning the study. For both groups receiving corticosteroids, the order of the doses was randomized. A few individuals were not tested at every dose; the number of subjects for each dose is indicated in Figs. 1 and 2.

Comparisons of neutrophilia with different agents. In a fourth group of 15 normal volunteers the PMN responses to hydrocortisone, prednisone, endotoxin, and etiocholanolone were compared. For this study, intravenous hydrocortisone (200 mg) and oral prednisone (40 mg) were given as indicated above.

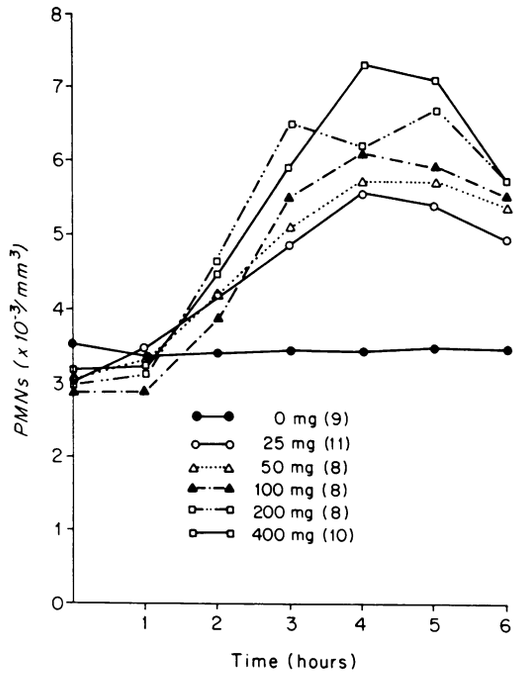

FIGURE 1 Responses of the PMN count to various doses of intravenous hydrocortisone. The points shown are mean values for groups of the sizes indicated in the parentheses.

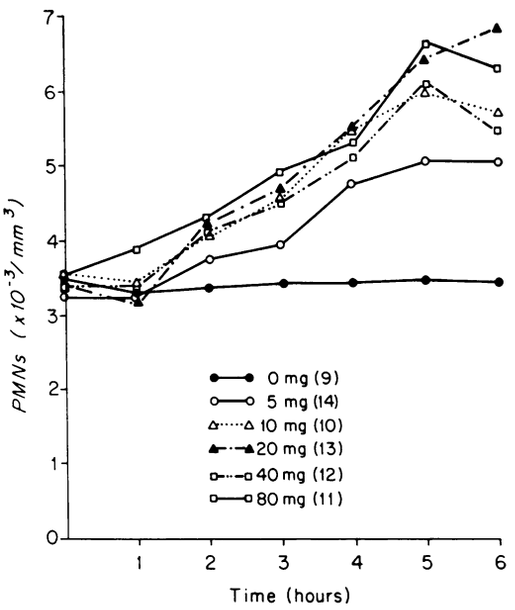

FIGURE 2 Responses of the PMN count to various doses of oral prednisone. The points shown are mean values for groups of the sizes indicated in the parentheses.

Endotoxin from *Salmonella abortus-equi* (Lipexal, Dorsey Laboratories, Lincoln, Nebr.) (0.8 ng/kg) was given intravenously at 8:00 a.m. as previously described (2) with PMN counts measured for 6 h. Etiocholanolone (prepared in propylene glycol by Pharmaceutical Development Service, Clinical Center, National Institutes of Health, Bethesda, Md.) was administered intramuscularly at 8:00 p.m. at previously reported (3) with PMN counts done before and 12, 15, and 18 h after administration. The responses to these four agents were measured 7 days apart in a variable, although not random, sequence. In two groups of patients, 7 patients with Wegener's granulomatosis on cyclophosphamide therapy and 10 patients with chronic idiopathic neutropenia, the PMN responses to the same doses of hydrocortisone, endotoxin, and etiocholanolone were compared. In these individuals the tests were done at least 2 days apart in a random sequence.

Blood cell counts. Total leukocyte counts were made on EDTA anticoagulated, antecubital vein blood samples using a Coulter Counter (model Fn, Coulter Electronics Inc., Hialeah, Fla.). Differential leukocyte counts were made on air-dried Wright's stained smears by either of two experienced technicians. In the normal subjects and patients without neutropenia (PMN counts greater than 2,000/mm³) 100 cell differential counts were done. 100 cells were counted because in non-neutropenic subjects the counting accuracy for PMNs, in contrast to the less numerous leukocyte types, is increased little by counting larger numbers of cells (21, 22). In the neutropenic subjects 200 or 400 cell differential counts were made routinely. PMNs were categorized as "mature" if the nuclear chromatin was clearly separated to lobes by any narrow strands of nuclear material.

Analysis of data. For the dose-response studies of hydrocortisone and prednisone, the areas under the individual response curves were calculated. The individual PMN responses were also determined by subtracting the base-line

TABLE I

Neutrophil Responses of Normal Volunteers to Various Doses of Hydrocortisone and Prednisone

Subject	Dosage				
Hydrocortisone					
	25 mg	50 mg	100 mg	200 mg	400 mg
1	3,202*	4,698	3,588	3,348	2,280
	5,284	2,308	6,177	7,299	6,602
2	2,400	3,159	3,135	3,172	2,912
	4,073	2,609	4,509	7,960	3,896
3	2,852	2,145	2,340	2,016	2,688
	1,228	3,284	2,390	4,681	5,766
Prednisone					
	5 mg	10 mg	20 mg	40 mg	80 mg
4	4,884	4,647	3,024	2,440	3,660
	1,752	3,039	3,658	2,852	4,060
5	3,380	4,352	4,422	4,240	3,300
	2,296	1,912	2,039	2,614	2,233
6	4,910	2,464	3,475	5,369	6,164
	3,689	2,666	3,470	4,024	4,740

* For each test upper number is base-line neutrophils/mm³, lower number is response, i.e., maximum value minus base line.

PMN count from the maximum count observed. Because there was a high correlation coefficient for the areas and the maximum count changes (e.g. for hydrocortisone, 200 mg, and prednisone, 40 mg, the Spearman rank correlation coefficients (23) were 0.93 and 0.73, respectively; $P < 0.01$), the difference between the base-line and maximum counts observed was used as an accurate measure of each individual response, as in previous studies (4, 8-15). Correlations of base-line counts and responses within groups of subjects were made using the Spearman rank correlation test, and group means were compared by Student's t test.

RESULTS

The dose-response relationships for intravenous hydrocortisone and oral prednisone showed that the maximum increases in counts occurred within 4–6 h. (Figs. 1 and 2). With increasing doses of either corticosteroid, there was a modestly upward trend in the mean PMN responses, measured either as the maximum change in the PMN counts or the areas under the response curves. The mean response to 5 mg of prednisone was significantly smaller than the mean response to the three highest doses of this agent ($P < 0.05$). Otherwise, there were no significant differences ($P > 0.1$) between the mean responses to the different doses of the same agent. The magnitude of the individual variability in these data is illustrated for six representative subjects in Table I.

Comparison of the four leukocytosis-producing agents in 14 normal subjects (Table II) showed that the mean PMN count changes with hydrocortisone, prednisone, and etiocholanolone were similar whereas endotoxin, in the dose employed, gave a significantly larger response ($P < 0.05$). One subject, number 8, had no response to etiocholanolone in this test or even when the test was later repeated (data not shown). In these tests none of these agents caused a consistent shift to nonsegmented PMNs. There was no significant correlation of the base-line PMN counts and PMN responses for any agent ($r < 0.37$, $P > 0.2$). Since the glucocorticosteroid responses for these 14 normal subjects were approximately normally distributed, 95% confidence intervals were calculated to determine the normal responses. The confidence interval for the maximum change in the PMN count was 1,600–6,800 PMNs/mm³ for hydrocortisone and 1,700–7,500 PMNs/mm³ for prednisone. The range of the responses was 2,600–6,400 PMNs/mm³ for hydrocortisone and 2,500–6,900 PMNs/mm³ for prednisone. The responses to endo-

TABLE II

Neutrophil Responses in Normal Subjects

Subject	Hydrocortisone 200 mg	Prednisone 40 mg	Endotoxin 0.8 ng/kg	Etiocholanolone 0.1 mg/kg
1	1,397*	1,944	1,976	2,457
	3,135	2,520	2,337	2,647
2	3,224	2,800	2,420	2,990
	3,183	4,485	5,634	2,340
3	2,671	3,696	2,538	2,268
	5,873	5,513	2,732	3,662
4	1,932	2,256	2,880	2,280
	2,828	6,862	4,668	4,184
5	4,504	2,627	2,900	4,818
	5,152	4,259	8,440	5,290
6	2,883	2,496	2,950	3,500
	2,619	5,500	5,800	5,500
7	5,428	3,228	3,024	3,388
	4,419	3,933	13,581	4,354
8	4,060	4,338	3,451	7,141
	4,608	2,963	8,202	0
9	2,507	4,111	3,510	3,916
	4,238	4,366	7,234	4,779
10	3,562	3,799	3,528	5,826
	6,406	2,497	10,484	5,038
11	3,762	2,961	3,540	3,472
	4,518	5,000	2,460	1,463
12	3,780	3,198	3,828	8,220
	2,498	6,792	2,997	5,278
13	5,650	5,150	5,670	6,550
	4,550	5,150	6,130	5,050
14	4,851	3,630	6,000	5,396
	5,007	4,946	4,165	4,281
Base line	3,586±336‡	3,302±234	3,443±303	4,444±571
Response	4,216±324	4,613±362	6,061±880	3,783±439

* For each test upper number is base-line neutrophils/mm³, lower number is response, i.e., maximum value minus base line.
‡ Values shown are arithmetic mean±1 SEM.

toxin and etiocholanolone were not normally distributed. There was a long upper tail for endotoxin and long lower tail for etiocholanolone. The range for these responses was 2,300–13,600 PMNs/mm³ for endotoxin and 0–5,500 PMNs/mm³ for etiocholanolone.

In a group of seven patients with Wegener's granulomatosis on stable doses of cyclophosphamide (25–100 mg/day), whose mean PMN counts were normal, the mean responses to all three leukocytosis-producing agents were reduced compared to normal ($P < 0.05$, t tests) (Table III). Using the 95% confidence value for the hydrocortisone response from the normal subjects, five of the nine hydrocortisone responses would be considered to be within normal limits. Three of the endotoxin responses were normal, by previously established criteria (1, 24), i.e., a PMN increase of more than 2,000/mm³. Only one of the etiocholanolone results was above the accepted lower limits of normal (3, 9), i.e., an increase of greater than 2,600 PMNs/mm³. As in the normal subject, there was no significant correlation of the base-line counts and responses for any agents ($r < 0.58$, $P > 0.05$).

TABLE III

Neutrophil Responses in Patients with Wegener's Granulomatosis on Cyclophosphamide

Patient	Hydrocortisone 200 mg	Endotoxin 0.8 ng/kg	Etiocholanolone 0.1 mg/kg
E. B.	3,500*	2,650	4,964
	2,221	1,482	935
S. C.	2,592	2,244	2,769
	1,966	1,422	2,211
	4,752	3,550	4,598
	448	454	560
L. D.	2,660	3,140	2,990
	2,180	1,020	1,060
P. D.	3,780	6,250	3,830
	4,820	2,250	2,070
M. G.	3,498	3,245	2,880
	1,374	2,917	3,435
J. T.	2,260	3,950	4,160
	1,180	1,900	1,488
G. W.	2,325	2,272	2,460
	1,635	2,028	2,105
	2,349	2,788	2,488
	1,011	1,802	1,054
Base line	3,080±284‡	3,343±409	3,459±315
Response	1,781±416	1,697±238	1,657±296

* For each test upper number is base-line neutrophils/mm³, lower is reponse, i.e., maximum value minus base line.
‡ Values shown are arithmetic mean±1 SEM.

In patients with chronic idiopathic neutropenia (stable PMN counts less than 2,000/mm³), all studied when clinically well, the mean base-line counts were significantly less than the base-line values of both the normal subjects and the patients on cyclophosphamide ($P < 0.05$) (Table IV). For this group of patients, the mean PMN responses for all three agents were significantly reduced compared to normal ($P < 0.05$, t tests). Etiocholanolone gave the smallest mean response, significantly smaller than the mean responses to endotoxin or hydrocortisone ($P < 0.005$). Of the three patients responding normally to endotoxin, two responded normally to hydrocortisone and the other value was borderline normal.

No side effects such as abdominal discomfort, bleeding, or mood changes were noted with these single doses of corticosteroids. In contrast, etiocholanolone regu-

TABLE IV

Neutrophil Responses in Patients with Chronic Idiopathic Neutropenia

Patient	Hydrocortisone 200 mg	Endotoxin 0.8 ng/kg	Etiocholanolone 0.1 mg/kg
C. B.	1,050*	700	295
	692	460	474
V. B.	198	279	260
	714	1,107	0
H. D.	390	580	186
	1,080	65	1,064
G. H.	425	550	736
	1,575	2,007	814
A. M.	318	382	504
	2,166	3,699	201
P. M.	250	350	630
	1,530	2,450	270
P. S.	468	118	242
	966	314	499
L. T.	438	235	405
	1,492	651	575
E. W.	36	28	27
	11	28	15
M. S.	176	112	340
	1,239	392	145
Base line	374±87**	333±70	362±67
Response	1,146±189	1,117±385	405±109

* For each test upper number is base-line neutrophils/mm³. lower number is response, i.e., maximum value minus base line.
‡ Values shown are arithmetic mean±1 SEM.

larly caused pain at the injection site lasting for up to 24 h, and endotoxin commonly caused slight fever and malaise as previously reported (2, 3).

DISCUSSION

The neutrophilic leukocytosis that follows administration of endotoxin (5), etiocholanolone (6), and hydrocortisone (7) has been attributed chiefly to mobilization of PMNs from the bone marrow reserves. The basis for this concept is that these agents can cause an increase in the number of nonsegmented blood PMNs (1, 3, 7), an influx to the blood of labeled marrow PMNs (6, 25), and a dilution of diisopropyl fluorophosphate ([^{32}P]DFP)-labeled blood PMNs by unlabeled, presumably marrow, PMNs (5, 7). In animal studies sufficient doses of endotoxin and glucocorticosteroids can be given to deplete blood and marrow PMNs (26, 27). In man the marrow mass of mature PMNs has been difficult to quantitate precisely, and the magnitude of the marrow PMN changes that these agents produce is probably too small to permit documentation of the egress of cells from the large marrow reserve pool with present techniques (28).

In addition to the accelerated release of marrow cells, several other factors contribute to the quantitative rise in the blood PMN count for each of these agents. The glucocorticosteroids, for instance, cause a decrease in granulocyte egress from the blood (7, 29) and a prolongation of the blood PMN half-disappearance time (5, 29). Endotoxin, on the other hand, causes blood granulocytes to be transiently shifted from the circulating granulocyte pool to the marginal pool, which leads to an apparent granulocytopenia shortly after its administration (5). Endotoxin, in contrast to glucocorticosteroids and etiocholanolone, causes the release of progressively higher numbers of nonsegmented PMNs at progressively higher doses, with no apparent plateau to this dose-response curve in man (1). At the doses usually given, however, no shift to nonsegmented cells normally occurs (13, 15). The time-course of the PMN response to endotoxin and the glucocorticosteroid is very similar with the peak response occurring after 4–6 h. Etiocholanolone causes a leukocytosis which develops much more slowly (3). After intramuscular injection of etiocholanolone, a local inflammatory response develops followed by a peaking of the blood PMN count 12–18 h later (3). The height of the PMN response is dose related (3). Thus, there are substantial differences in the mechanisms whereby each of these agents raise the blood PMN count.

Despite these overall differences in the characteristics of the granulocytosis, the present study was undertaken to determine if the PMN count changes after administration of these agents are sufficiently similar to permit the use of glucocorticosteroids instead of endotoxin or etiocholanolone to measure the marrow granulocyte reserve response. To establish the dose of corticosteroids to be used, the dose-response relationship was examined. There were relatively small differences in the responses with increasing doses for both corticosteroids. Therefore, somewhat arbitrarily, a single dose of 200 mg of hydrocortisone and 40 mg of prednisone were chosen for comparison with the usual test doses of endotoxin and etiocholanolone (3). In this comparison, endotoxin gave a larger mean response than the other agents at a dose that did not produce a shift to nonsegmented PMNs (1). It should be noted that these endotoxin responses are not normally distributed (Table II); two individuals gave particularly large responses. This individual variability in the PMN response to endotoxin has been noted previously (1, 15). In fact, because of this variability, the minimum response after endotoxin considered as "normal" (an increase of at least 2,000 cells/mm^3) has been based on the range of results in normal subjects rather than a calculated confidence interval. For etiocholanolone, the basis of the nomal response also has been the range of responses in a group of normal subjects (3). In the present study, two individuals had responses below this previously defined lower limit of normal.

The responses to both hydrocortisone and prednisone in this study were somewhat less variable than for endotoxin and etiocholanolone as reflected by a smaller SEM response. These responses also were nearly normally distributed, permitting calculation of a 95% confidence interval for the normal response. This normal response was 1,600–6,800 PMNs/mm^3 for hydrocortisone and 1,700–7,500 PMNs/mm^3 for prednisone.

In the two patient groups studied, the mean responses to each of the three test agents were reduced. In the patients with chronic idiopathic neutropenia, etiocholanolone gave a significantly smaller mean response than hydrocortisone or endotoxin. The reason for this difference is unclear. It is possible that the local inflammatory response is less in patients with such severe neutropenia leading to a proportionally smaller response. Otherwise, in the individual patients there was reasonably good agreement of the tests. In the chronic neutropenic group, the same three patients had their highest responses to hydrocortisone and endotoxin. In the patients with Wegener's granulomatosis, the same patient had very low responses to all three tests. Thus, these results indicate that the PMN response that occurs in these patient groups can probably be measured equally well with any of these three agents.

In these patients and normal volunteers, no side effects were noted with the 152 glucocorticosteroid doses

administered. In contrast, etiocholanolone regularly caused at least some discomfort at the injection site, and malaise and fever occurred after endotoxin. The glucocorticosteroids thus appear to have several advantages over endotoxin and etiocholanolone for measuring the neutrophil reserve response: (*a*) greater availability, (*b*) more predictable response in normal subjects, (*c*) equivalent responses in patient studies, and (*d*) fewer side effects.

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