DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 11(11), 1939-1945 (1985)

LYMPHOCYTOPENIC EFFECT OF PREDNISOLONE AS A MEASURE OF FORMULATION EFFICACY

H.Y. Abdallah, N. Khalafallah and Said A. Khalil,
Department of Pharmaceutics, Faculty of Pharmacy,
University of Alexandria, Alexandria, Egypt

ABSTRACT

The lymphocytopenic effect of prednisolone was measured and investigated as a possible index of formulation efficacy. Blood samples were collected by finger puncture in 10 volunteers two hours after the administration of 10 mg of prednisolone. Two tablet formulations with marked difference in dissolution behavior were used. Differential leukocytic counts were made on blood films, suitably stained. The results obtained indicated a measurable decrease in the lymphocytic count in each volunteer two hours after drug administration. The reduction in lymphocytic counts produced was in accordance with the dissolution data. The highly significant inter-treatment difference indicated that measurement of the selected pharmacologic effect afforded good discrimination between the slow and fast dissolving formulations.

INTRODUCTION

Glucocorticoids are commonly employed in therapy for their antiinflammatory and immunosuppressive effects. These effects cannot be quantitatively measured. The

0363-9045/85/1111-1939$3.50/0

assessment of therapeutic effectiveness of oral corticosteroid formulations has, therefore, followed the trend of bioavailability determination by blood level monitoring [1]. In search for a simple and sensitive *in vivo* method for testing the efficacy of prednisolone tablet formulations, we have investigated the possible use of a one-point lymphocytopenic effect determination.

EXPERIMENTAL

Two, 5 mg, prednisolone tablet formulations with marked difference in their dissolution behavior were tested. Treatment A tablets were prepared by direct compression of prednisolone (Upjohn)-microcrystalline cellulose (Avicel PH 101, FMC Corp.) 1:10 trituration ($t_{60\%\ \text{dissolution}}$ = 30 min.). The steroid was dissolved in methanol and deposited while stirring on the cellulose by evaporating at room temperature. Drying and sieving preceded direct compression. A market tablet product (Deltacortril, Pfizer Misr, batch no. 12596001) served for treatment B ($t_{60\%\ \text{dissolution}}$ = 44 min.).

Ten volunteers, with no evidence of adrenal insufficiency or hyperfunction, participated in this study. Subjects were asked to abstain from taking any medication for a period of one week prior to each experiment. After an overnight fast, each subject swallowed two prednisolone tablets, randomly from treatment A or B, with 100 ml of water and received the other treatment one week later. Blood samples were obtained by finger puncture at zero time and two hours after drug administration during which the subjects abstained from eating.

A drop of blood was spread into a thin, one-cell thick film on a microscope slide and left to dry in air. The film was then stained with a 0.1% methanolic solution of Leishman stain (Prolabo, France) for three minutes. The stain was diluted with water and set aside for seven

minutes. The film was washed under running water and left to dry in air. Differential leukocytic counts were carried out by counting the relative numbers of the different types of leukocytes in adjacent fields covering a total of 100 leukocytes.

RESULTS

The differential lymphocyte counts before, and two hours after administration of 10 mg of prednisolone in treatments A and B are given in Table 1. The zero-hour counts were within the normal range reported for lymphocytes (15-60%, ref. 2). Administration of prednisolone in the A and B treatments resulted in a measurable decrease in the lymphocytic count in each volunteer two hours after drug administration. Percentage reduction ranged from 16.3-88.5% for treatment A and from 6.6-48.0% for treatment B. The observed reduction was statistically significant in both treatments (t values, 0 *vs* 2 hr, Table 1) and indicated a fairly high sensitivity of the measured pharmacologic response to the dose administered.

The high initial lymphocytic counts permitted detection of varying intensities of response two hours after prednisolone administration. Figure 1 indicates that the reduction in lymphocytic counts produced in treatment A was greater in all subjects compared to B and was in accordance with the dissolution data. The highly significant intertreatment difference (t values, A *vs* B, Table 1) indicated good discrimination between the slow and fast dissolving formulations.

DISCUSSION

The present study provides preliminary data on the possible use of the lymphocytopenic effect of prednisolone as a parameter indicating formulation efficacy. This

TABLE 1

Differential Counts of Lymphocytes Before, and Two Hours After Oral Administration of 10 mg Prednisolone to 10 Volunteers.

Subject	Treatment A			Treatment B		
	0hr.	2hr.	% reduction	0hr.	2hr.	% reduction
1 (M,41,82)[a]	43	36	16.3	40	34	15.0
2 (M,25,65)	51	23	54.9	59	35	40.7
3 (M,27,77)	48	32	33.3	51	46	9.8
4 (M,25,82)	55	26	52.7	57	52	8.8
5 (M,27,72)	43	23	46.5	35	26	25.7
6 (M,35,78)	50	34	32.0	70	57	18.6
7 (M,38,75)	41	22	46.3	30	22	26.7
8 (F,40,55)	26	3	88.5	25	13	48.0
9 (F,26,57)	56	34	39.3	61	57	6.6
10 (F,26,70)	48	38	20.8	42	37	11.9
Mean[b] (SE)	46.1 (2.7)	27.1 (3.3)	43.1 (6.5)	47.0 (4.7)	37.9 (4.8)	21.2 (4.4)
t value 0 vs 2hr	8.5 (>99.9%)[c]			4.7 (>99.5%)		
t value A vs B	5.1 (>99.9%)					

a. Sex, age, body weight.
b. Standard error of the mean.
c. Significance level.

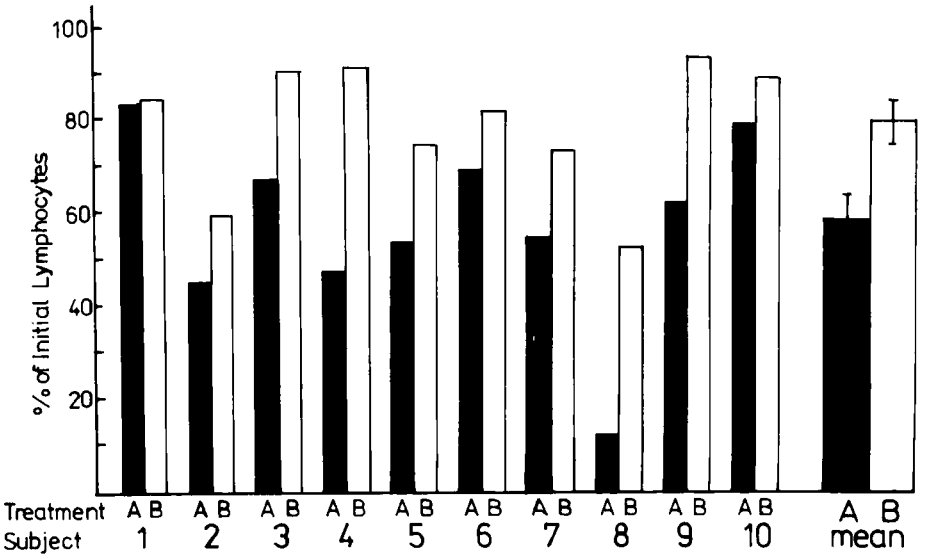

FIGURE 1

Lymphocytic Counts Two Hours After Oral Administration of 10 mg Prednisolone to Volunteers According to Treatments A and B. Bars Denote the Standard Error of the Mean.

approach was encouraged upon reviewing the pharmacodynamic profile of the lymphocytopenic effect in relation to the therapeutic effects of prednisolone.

Associated with the antiinflammatory and immunosuppressive effects of prednisolone and other corticosteroids are changes in the lymphoid tissue which are characterized by decreased tissue mass as well as dissolution and decreased production of lymphocytes, hence the term lymphocytopenia [3]. The lymphocytopenic effect of different corticosteroids closely parallels their potency [3]. In case of prednisolone, the lymphocytopenia has been considered indicative of the intensity and duration of the antiinflammatory and immunosuppressive effects of the drug [4]. It was, therefore, felt that measurement of the lymphocytopenic effect of prednisolone

could provide direct information about the therapeutic effectiveness of its formulations. Endogenously secreted hydrocortisone can also produce the same effects on lymphocytes. However, the quantity required to produce such effects is much greater than that normally secreted by the adrenal cortex [5].

Concerning the reported time course of the lymphocytopenia resulting from prednisolone administration, the effect is of a dynamic nature as demonstrated by the immediate change in the lymphoid tissue [3]. Following a single dose of prednisolone, given orally or intravenously, the lymphocytopenic effect occurs one hour later and reaches a maximum in 2-4 hours. The effect persists for 6-12 hours [3] and may still be detected 72 hours after drug administration [4]. In the present study, the selected pharmacologic effect was measured at its reported peak time of 2 hours. This was considered sufficient to address the question of whether or not measurement of lymphocytopenia can provide information on the relative efficacy of prednisolone formulations.

It is felt that the approach of monitoring the activity of prednisolone formulations through measurement of the lymphocytopenic effect is worthy of further investigation. The ease of performing blood counts and the lack of inconvenience to voluteers were notable advantages. Establishment of quantitative correlation between the degree of lymphocytopenia and the administered dose will be of value in this respect.

REFERENCES

1. A.V. Tembo, M.R. Hallmark, E. Sakmar, H.G. Bachman, D.J. Weidler and J.G. Wagner, J. Pharmacokin. Biopharm., 5, 257 (1977).
2. V.B. Dacie and S.M. Lewis, "Practical Hematology", 5th ed., Churchill Livingstone, Edinburgh, U.K., 1975, p.13.

3. G. Sayers and R.H. Travis in "The Pharmacological Basis of Therapeutics", 4th ed., L.S. Goodman and S. Gilman, eds., Macmillan Co., New York, N.Y., 1970, p. 1622.
4. W.J. Jusko and J.Q. Rose, Ther. Drug Monit., 2,169 (1980).
5. A.G. Guyton, "Textbook of Medical Physiology", 3rd ed., Saunders Co., Philadelphia, 1966, p. 133.