Corticosteroids in Endotoxic Shock (35928)

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Endotoxic shock is still one of the most lethal forms of shock encountered in clinical practice although considerable advances have been made in its treatment. While the high mortality rate is in part due to the nature and often inevitable deleterious effects of the underlying disease, judicious use of various available therapeutic agents can reduce mortality. Among these are the corticosteroids, which have undoubted ability to markedly increase survival rate experimentally when there is no underlying disease to affect the outcome (1). In recent years, there has been a definite tendency to use higher doses of steroids and undoubtedly large pharmacologic doses are required. The studies of Weil and Allen (1) indicated the importance, at least experimentally, of administration of corticosteroids in the period 2 hr before to 15 min after injection of endotoxin for maximum protective effect. Earlier or later treatment was significantly less effective or ineffective depending on the time interval. These authors also found that the effect of prednisolone was definitely dose related, maximal protection occurring at 120 mg/kg. Smaller doses were less effective and larger doses were inherently lethal causing almost 100% mortality at a dose level of 960 mg/kg. Because of the critical dose relationships and the more frequent use of larger doses in therapy, it seemed desirable to obtain further information regarding the therapeutic efficacy of corticosteroids, and to compare three frequently used steroids to determine if the ED_{50} values were significantly different.

Methods. Purified *E. coli* 055 B5 Bacto lipo-polysaccharide B endotoxin, control No. 522131 was obtained from Difco Laboratories. Young white Swiss mice weighing 20–25 g were used. Initially the approximate LD_{80} was determined by giving various doses of endotoxin dissolved in 0.2 ml of 0.9% NaCl, ip, to groups of 10 mice at each dose level. The 72-hr mortality rate was determined and the dose to be used in the subsequent experiments was calculated to be 56 μg/g. Subsequently, during the main part of the experiment, 250 control mice were injected intraperitoneally with the endotoxin alone in a volume of 0.2 ml of NaCl. In these 250 mice, the average mortality rate was 83.2%. This agreed closely with the average mortality rate of 83.0% in the 270 animals receiving the lowest dose of each steroid.

To determine the effect of corticosteroids on mortality rates, 2700 mice injected with the same amount of endotoxin, were treated either with dexamethasone, prednisolone, or hydrocortisone at one of 10 dose levels, to yield a total of 90 mice at each dose level of each steroid (Table I). In these, a concentrated solution of the steroid was serially diluted with the endotoxin solution in such a way that the final dose of endotoxin was 56 μg/g in 0.2 ml of NaCl solution with the desired dose of steroid. This was given ip as in the control animals. All steroids were the disodium phosphate salts to minimize differences in absorption, but doses were corrected to and reported in terms of the molecular weight of the parent compound, *i.e.*, dexamethasone, 392.45; hydrocortisone, 362.51; and prednisolone, 360.44. The animals were followed for 72 hr, as the preliminary studies showed that death rarely occurred after this interval, in either the control or treated groups. Survival rates were determined at 24, 48, and 72 hr, but most of the fatalities occurred within the first 48 hr.

Results. Table I shows a summary of the 24, 48, and 72 hr survival data. In order to estimate relative potencies and ED_{50}, all 72 hr survival rates were adjusted for "natural"

TABLE I. Effect of Corticosteroids on Survival Rate from a Standard Dose of Endotoxin.

Dexamethasone					Hydrocortisone					Prednisolone				
	Survival rate (%)					Survival rate (%)					Survival rate (%)			
Dose (μg/g)	24 hr	48 hr	72 hr	Adjusted[a]	Dose (μg/g)	24 hr	48 hr	72 hr	Adjusted[a]	Dose (μg/g)	24 hr	48 hr	72 hr	Adjusted[c]
0.2	41.1	32.2	25.6[b]	0	2	41.1	23.3	26.1[b]	0	1	47.8	36.7	34.4[b]	11.4
0.4	53.3	37.8	34.4	11.4	4	50.0	37.8	36.7	14.5	2	52.2	37.8	33.3	9.9
0.8	47.8	41.1	40.0	18.9	8	38.9	27.8	27.8	2.4	4	55.6	37.8	36.7	14.5
1.6	55.6	44.4	41.1	20.4	16	42.2	37.8	35.6	13.0	8	56.7	43.3	42.2	21.9
3.2	63.3	47.8	42.2	21.9	32	52.2	38.9	36.7	14.5	16	64.4	53.3	53.3	36.9
6.4	68.9	56.7	53.3	36.9	64	73.3	63.3	61.1	47.4	32	70.0	51.1	48.9	30.9
12.8	75.6	67.8	66.7	55.0	128	83.3	73.3	70.0	59.5	64	70.0	55.6	52.2	35.4
25.6	74.4	67.8	65.6	53.5	256	81.1	67.8	67.8	—[c]	128	81.1	73.3	71.1	60.9
51.2	82.2	76.7	74.4	65.4	512	62.2	41.1	40.0	—[c]	256	90.0	81.1	78.9	71.5
102.4	87.8	78.9	75.6	67.0	1024	8.9	7.8	7.8	—[c]	512	73.3	53.3	52.2	—[c]

[a] Adjusted or "natural" survival rate (selected at 26%) by least squares fitted probit line.

[b] Response to these doses were combined and added to controls to estimate the "natural" survival rate.

[c] Represents toxic effect and omitted from estimate of ED_{50}.

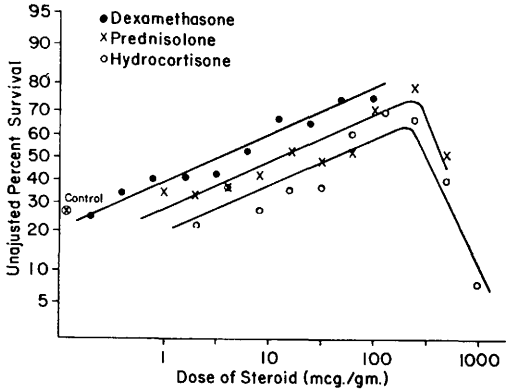

FIG. 1. Unadjusted survival curves, see text.

survival. This was selected by the statistician at 26% from the survival rates for the controls and the smallest dose of dexamethasone and hydrocortisone. In addition, the 3 highest doses of hydrocortisone and the highest dose of prednisolone were omitted since these showed toxicity. The data were evaluated by a maximum likelihood procedure using probit analysis, adjusted for "natural" survival. There was some evidence of heterogeneity and lack of parallelism due mainly to the somewhat more erratic behavior of hydrocortisone. However, this was not statistically significant and the slopes were considered to be parallel within the limits of experimental error.

Figure 1 shows free-hand curves drawn through the unadjusted data, and Fig. 2 shows the least squares fitted probit lines for the survival data adjusted for "natural" survival. The individual slopes were found to be parallel within the limits of experimental error, and the common slope for all three steroids was found to be significant at $p<0.01$.

Table II summarizes the estimates of relative potency, ED_{50}, and 95% confidence limits. Dexamethasone was the most potent steroid, followed by prednisolone and then by hydrocortisone. The relative potencies were significantly different at $p<0.01$. Based on the survival data adjusted for "natural" survival, the ED_{50} for dexamethasone was 18.6 μg/g; for prednisolone, 70.9 μg/g; and for hydrocortisone, 146 μg/g. The LD_{50} values for the unadjusted data were approximately $\geqq$ 200 μg/g for dexamethasone, about 500 μg/g for prednisolone, and about 375 μg/g for hydrocortisone. Thus, the therapeutic indexes determined from the adjusted ED_{50} and the unadjusted LD_{50} were estimated approximately as follows: dexamethasone $\geqq$ 200/18.6 $\geqq$ 11; prednisolone 500/70.9 = 7.1; and hydrocortisone 375/146 = 2.6. However, the toxicity data were not considered sufficient to estimate the LD_{50} and the therapeutic indexes for all three steroids in order to carry out statistical tests.

An estimate of the dose of each steroid at which a protective effect began to occur was also made. Based on the binomial distribution for samples of 90, the upper limit of survival at $p = 0.05$ for the adjusted data is about 3.3%. The estimates of the $ED_{3.3}$ and 95% confidence limits were as follows: dexamethasone, 0.1 μg/g (0.04, 0.4); hydrocortisone, 1.0 μg/g (0.3, 2.9); and prednisolone 0.5 μg/g (0.2, 1.5). However, since estimates in the tails of the distribution are notoriously inaccurate and rely heavily on the assumption of normality, these estimates should only be used as a guide and in a relative sense.

The dose of each steroid above which no *statistically* significantly greater protection occurred was also determined. This was carried out after making an arc sine transformation of the percentage of survivals. The analysis was carried out by comparing the response for the lowest dose tested to the average response for the higher doses tested (excluding the responses to the highest doses that showed obvious evidence of toxicity). If this showed a significant difference, the test

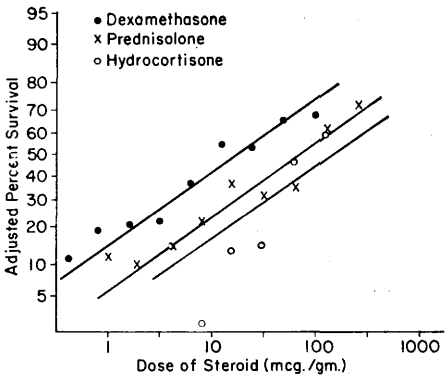

FIG. 2. Adjusted survival curves; see text.

TABLE II. Relative Potencies, ED_{50}, and 95% Confidence Limits.

Potency of	Relative to Dexamethasone	Relative to Hydrocortisone	Relative to Prednisolone
Dexamethasone	1	7.8 (4.6, 13.4)[c]	3.8 (2.4 , 6.0)[c]
Hydrocortisone	0.13 (0.07, 0.22)	1	0.49 (0.28, 0.83)
Prednisolone	0.26 (0.17, 0.41)	2.1 (1.2, 3.6)[b]	1
ED_{50}[a] (μg/g)	18.6 (12.2 , 28.6)	146 (83.2, 257)	70.9 (44.9 , 112)

[a] Based on combined slope and adjusted for "natural" survival rate.
[b] Statistically significantly more potent: ($p < 0.01$); [c] ($p < 0.001$).

was repeated comparing the response for the next higher dose to the average response for the higher doses. This procedure was continued until no significant difference was found. Prednisolone gave an anomalous result at 16 μg/g, since the next higher dose (32 μg/g) was found to give a response significantly lower than the average response to the higher doses. Therefore, for prednisolone, the test was allowed to continue. By this method the optimum protective doses were for dexamethasone, 12.8 μg/g; for hydrocortisone, 64 μg/g; and for prednisolone, 128 μg/g.

Discussion. These results confirm the efficacy of corticosteroids in reducing the mortality rate of experimental endotoxic shock, at least in these circumstances, *i.e.*, a single challenge with endotoxin without clinical infection or complicating disease, and with simultaneous administration of steroid. There have been no definitive studies done to explain the basic mechanism of action of corticosteroids in endotoxic shock, but hemodynamic studies in dogs, carried on concurrently, show that large doses of hydrocortisone are definitely protective, and that the fall in cardiac output, arterial blood pressure, and regional blood flow are less than in the control animals. Since of the three steroids tested, dexamethasone had the highest therapeutic index, and since the highest doses of hydrocortisone and prednisolone were inherently lethal, dexamethasone would appear to be the steroid of choice among these three. Again, the basic mechanism for the lethal effects of higher doses of hydrocortisone and prednisolone is unknown, but it is of some interest to note that this effect began to occur at about the same dose level for each steroid, *i.e.*, approximately 500 μg/g. Since the maximum dose of dexamethasone was only 102.4 μg/g, it is unknown if similar lethal effects would have occurred with doses of 500 μg/g or above of dexamethasone.

Whether or not other potent synthetic steroids not tested would have significant advantages was not determined. However, since the data do suggest that the maximum survival rate at the optimum dose of each steroid was approximately the same, and since dexamethasone showed no apparent evidence of toxicity, this possibility seems unlikely.

One can only speculate as to the relative value of corticosteroids in human endotoxic shock. Because of species differences and the complicated pathophysiology usually seen in humans with the disease, efficacy may not be the same as in experimental studies. Nonetheless, the following conclusions are probably warranted. Corticosteroids should be used with other indicated therapy in the treatment of moderate to severe endotoxic shock as they have a very significant effect experimentally in increasing survival rate. Although it is difficult to specify the exact dose for use in humans, the data indicate that large doses are required for an optimlm effect and the doses should probably be higher than those customarily used. The data of Weil and Allen (1) indicate that early therapy is important, but since the exact mechanism of steroid action in endotoxic shock is not well defined and since in the human situation there is usually continued liberation of endotoxin at least for awhile, corticosteroid therapy should be tried even though the time of initiation is later in the course of the disease.

Summary. Hydrocortisone, prednisolone, and dexamethasone given simultaneously with endotoxin were found to have a significant protective effect in mice, reducing mortality rate from 83% in control groups to about 20–25% in treated groups at optimum dose levels. Dexamethasone had the highest therapeutic index, as hydrocortisone and prednisolone were lethal at the higher dose levels.

1. Weil, M. H., and Allen, K. S., *in* "Inflammation and Diseases of Connective Tissue" (L. C. Mills and J. H. Moyer, eds.), p. 768. Saunders, Philadelphia (1961).

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