Evaluation of Infection-Enhancing Activity of Modified Corticoids.*† (25593)

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Although the effects of corticoids concerning inflammation, glycogen deposition and sodium retention are commonly investigated, the important side-effects on infection(1) have not been studied systematically. It will be shown here, that by corticoid treatment in hamsters, acquired resistance to *Besnoitia*, an obligate intracellular protozoan(2), is easily abolished, resulting in fatal relapse of chronic infection. The speed with which relapse occurs is proportional to dose of corticoid employed. Infection-enhancing activity of a number of modified corticoids has been evaluated by means of this system.

Materials and methods. A. *Infection. Besnoitia jellisoni* was originally isolated from white-footed mice, *Peromyscus maniculatus artemisiae*, found naturally infected at upper Salmon River, Idaho(2). Cyst-forming capacity of the organism was eliminated by rapid passage (twice weekly) in peritoneal cavity of mice. The strain is now beyond its 550th passage. Six to 8 week old Golden hamsters, *Mesocricetus auratus*, of random-bred stock, are infected subcutaneously with peritoneal exudate containing a few thousand *Besnoitia*. To permit development of resistance with persistence of infection (premunition), hamsters are treated for 4 weeks with sodium sulfadiazine in drinking water, at 90 mg% for females and 180 mg% for males. (Fed on Purina Lab Chow only, at temperatures 70-80°F, adult female hamsters drink approximately 10 ml daily, but males drink only 5-6 ml.) They are tested for resistance by reinfection, subcutaneously, and virtually all animals survive, although they may develop endophthalmitis(3) and signs of chronic encephalitis(2). After another 3 or 4 weeks they are ready for assay of corticoids; only those few animals showing progressive encephalitis with weight loss are eliminated from the test. For comparative experiment *Toxoplasma gondii* (RH strain) is used in similar manner to produce chronic infection in hamsters. As a rule sulfadiazine treatment has to be given intermittently for 2-3 months till chronic latency has been achieved(4). B. *Steroids.* The following corticoids were used (numbers and abbreviations refer to Fig. 2): 1. cortisone acetate (Eac); 2. hydrocortisone (F); 3. prednisolone (Δ^1F); 4. prednisolone acetate (Δ^1 Fac); 5. 2-methyl-9-α-fluoro hydrocortisone acetate (2Me 9F Fac); 6. 6-methyl prednisolone (6 MeΔ^1 F); 7. 16-α-hydroxy-9-α fluoro-prednisolone (triamcinolone, 16 OH 9FΔ^1 F); 8. 9-α fluoro-prednisolone (9 FΔ^1 F); 9. triamcinolone acetonide (acetaldehyde derivative, 16 OH 9 FΔ^1 F actnd); 10. 16-α methyl-9-α fluoro-prednisolone (dexamethasone, 16 Me 9FΔ^1 F). Additional steroids are listed in Table I. C. *Tests.* After grinding in ground glass homogenizer, compounds were administered suspended in aqueous vehicle containing .5% sodium carboxy-methylcellulose, .4% polysorbate 80 (Tween), .9% sodium chloride and .9-1.5% benzyl alcohol. However, Δ^1 Fac was used as supplied in aqueous vehicle containing .5% phenylethyl alcohol and .01% benzalkonium chloride. Graded doses at 2-4 levels were injected subcutaneously, once daily, 6 times weekly. Generally 4-6 hamsters were used/dose level; identical groups in repeated tests were combined for computation of means. Fatal relapse results from confluent pneumonia with focal necrosis or from acute encephalitis. Commonly, there is proliferation of *Besnoitia* at sites of corticoid injection(5); the number of deposits provides an estimate of drug insolubility. Specificity of lesions is checked by impression smears, which

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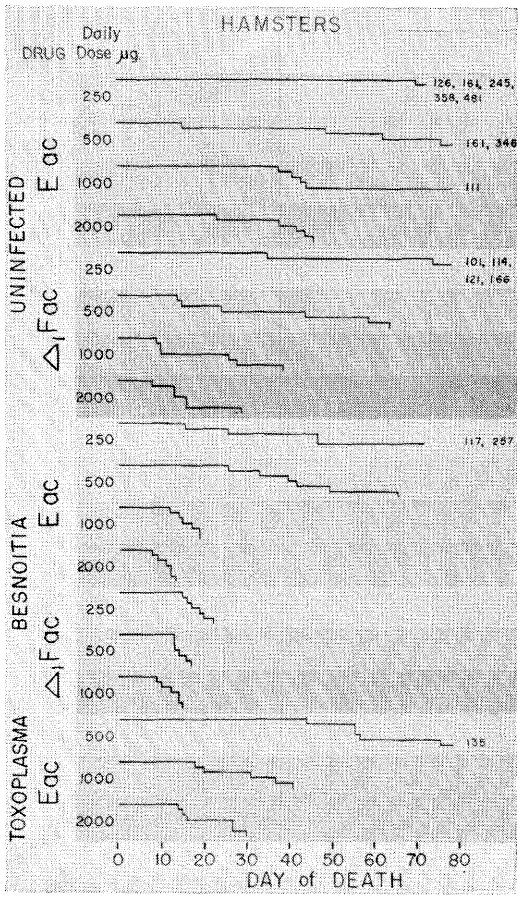

FIG. 1. Effects of cortisone (Eac) and prednisolone (Δ^1Fac) on uninfected hamsters and on hamsters with chronic *Besnoitia* and *Toxoplasma* infection. Ordinates represent No. of animals (out of 6) surviving at each dose level. Note rapid and uniform mortality in hamsters with *Besnoitia* infection and treated with large doses of either corticoid. Also note that lower doses of corticoids will result in death at irregular and widely dispersed intervals (day of death beyond 80 given in numerals).

show numerous crescentic *Benoitia* organisms, visible at magnifications of 200-500, following Giemsa staining, clearing and mounting with a coverslip.

Results. A. *Corticoid sensitivity of normal hamsters, and of hamsters with chronic* Besnoitia *or* Toxoplasma *infections (Fig. 1).* It is apparent that infected hamsters are more sensitive to corticoids than uninfected ones. Administration of 2000 μg of Eac daily resulted in death after an average of 11 days for *Besnoitia*-infected, 22 days for *Toxoplasma*-infected and 35 days for uninfected hamsters. Resistance to *Besnoitia* was consistently more sensitive to cortisone than resistance to *Toxoplasma.* In similar doses, Δ^1 Fac was more effective than Eac in bringing about death of hamsters, whether infected with *Besnoitia* or not. Specific organisms could generally be found in the lung or brain lesion of infected hamsters. However, when death was delayed beyond one month, other lesions often occurred, such as caused death of "uninfected" animals. These lesions included: phagedenic ulcers (30%) originating from bites, sites of corticoid injection, or from large brown fat pads; enteritis with peritonitis (20%); meningitis (9%); liver or spleen abscesses (9%); and dissecting aortic aneurysms that had ruptured (11%). Bacteria that were cultured included coliforms, *Proteus, Mima, Staphylococcus* and diphtheroids. With higher corticoid dosages relapses of *Besnoitia* infection occurred within a few days in second or third week. It is this period that appeared to be most useful for assay purposes.

B. *Dose-response curves for natural and modified corticoids and derivation of potency ratios (Fig. 2).* The mean days of death at

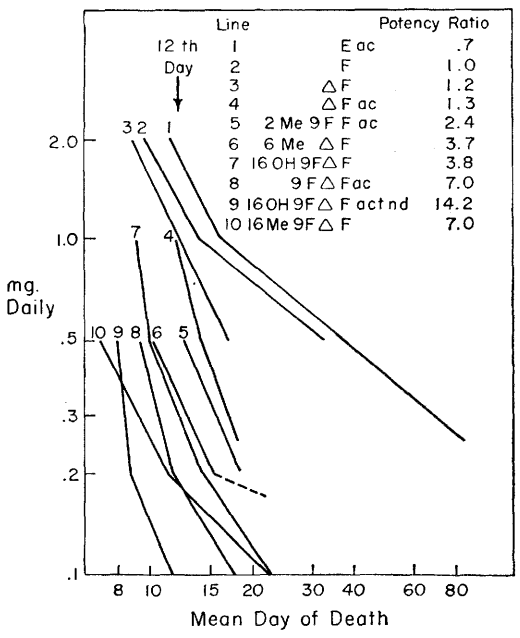

FIG. 2. Relapse of chronic *Besnoitia* infection in hamsters. Dose-response lines for 10 corticoids are identified by numbers. Both dose and time are charted logarithmically. Potency ratios for 12th day are given in inset.

TABLE I. Potency of Corticoids Relative to Enhancement of *Besnoitia* Infection in Hamsters. Tissue solubility was estimated from the number of steroid deposits remaining.

Steroids	Enhancement of infection	Solubility in tissue comp. to Eac
DOC (21-OH progesterone)	0 *	+ ¶
2-Me-11-OH "	0 †	-- **
9-F-11-OH "	0 ‡	--
6-Me-11-keto- "	.6	±
6-Me-17-acetoxy- " (Provera)	0 ‡	--
9,21-F-21-desoxy F	~ 1 §	--
9,21-F-21-desoxy Δ^1 F	~ 2 §	+
Eac	.7	±
Δ^1 Eac	.8	±
F	1	+
Δ^1 F	1.2	+
Δ^1 Fac	1.3	--
9-F Fac	3.1	±
9-F Δ^1 Fac	7.0	++
2-Me F	~ .4	--
2-Me 9-F Fac	2.4	--
6-Me Fac	1.5	+
6-Me Δ^1 F	3.7	+++
16-OH-9-F F	1.6	±
16-OH-9-F Δ^1 F	3.8	+
" acetnd	14.0	+++
16-Me 9-F Δ^1 F	7.0	+++

* Enhancement of infection tested up to 1 mg/day.
† Tested up to 1.5 mg/day.
‡ Tested up to 2 mg/day.
§ Steep curves, not intersecting 12th day.
‖ Active at 1 mg, not at 200 μg.
¶ +, greater solubility than Eac.
** --, lesser solubility than Eac.

2-4 dose levels were plotted logarithmically. Dose of drug that produced fatal relapse on 12th day (mean) was chosen for comparison of potency ratios. For hydrocortisone alcohol this is 1.3 mg (line 2) which serves as standard unit. Relative potency = 1.3/(mg of assayed corticoid required to produce death on 12th day). Ratios of enhancement of infection by all steroids tested are compared, and indications of drug solubility in tissue are given (Table I).

Discussion. A. *Model.* Hamsters are the most corticoid-sensitive small laboratory animal available (unpublished observations), being more susceptible to corticoid "toxicity" than mice, rats, and guinea pigs(6). Fig. 1 relates corticoid sensitivity of infected and control hamsters, with 73% of deaths even in the latter group accounted for by infection with indigenous flora. Similar to man, hamsters secrete hydrocortisone, unlike rats and mice that secrete corticosterone predominantly.

Chronic, latent *Besnoitia* infection is comparable to chronic tuberculous and fungal infections of man, which may either relapse, or be accentuated in their course by corticoid therapy if doses beyond the physiologic range are used. Acute and subacute *Besnoitia* infection is even more sensitive to corticoids than chronic infection utilized here(5). This infection system, which has been used for assay of compounds less potent than cortisone (7), may be representative of those acute viral infections, such as chickenpox and poliomyelitis, which are affected adversely by large doses of corticoids(1).

The action of corticoids on certain acute bacterial infections has been studied qualitatively(8,9,10) and quantitatively(11). However, excessive inocula resulting in rapidly fatal course were employed. Endotoxin effect, where present, may actually be moderated by corticoids. There is uncertainty as to which of a variety of intra- and extracellular factors affecting phagocytosis, digestion, or antibody mechanisms, are modified by corticoids, or whether their effect is on non-specific resistance or acquired immunity, which is unaffected in salmonellosis(13). In chronic *Besnoitia* infection organisms persist intracellularly, antibody is present (unpublished observations), and hamsters were shown to be immune to challenge. When pharmacologic doses of corticoids are given, organisms again start to multiply within cells that could previously curb this multiplication. Relapse can prove fatal to the host in approximately the same time as fatality results from primary acute infection not treated with sulfadiazine. A further indication of the corticoid-sensitivity of the hamster's resistance to *Besnoitia* is that chronic infection frequently leads to progressive and ultimately destructive adrenal involvement, apparently dependent on the endogenous hydrocortisone produced(5). Corticoid treatment did not affect stable antibody levels(14). Since hydrocortisone does not enhance proliferation of *Besnoitia* in non-immune hamsters, nor in hamster cell cultures (unpublished), and exerts its effect only after the presence of immunity had been indicated

by resistance to challenge, a direct growth stimulation in the complete host, or an effect on natural resistance alone, appears unlikely.

B. *Comparison of infection-enhancing effects.* By definition the Potency Ratios are based on amount of corticoid required to produce death on 12th day. They have only a relative meaning, but are fairly consistent in terms of rank relationship. Type of curves obtained depends not only on structure of corticoid, but also on biological half-life, especially solubility and rate of degradation. 16-hydroxylated and methylated compounds have been reported to be more active by mouth than by subcutaneous administration(15). The latter route was used throughout this study.

A 50% mortality end point (LD_{50}) in the ordinary sense is difficult to derive, since mortality occurs as a continuum, and there is no meaningful break-off point (Fig. 1). If one plots mean days of death for a wide dose range of corticoids, one obtains a sigmoid curve (hyperbolic tangens). Asymptotes are present near the maximal obtainable drug effect (Fig. 2), limited by speed of biological responsiveness, as well as near minimal observable effects, approaching natural longevity. Near central part of curves parallelism could be observed. Usually, assays are conducted in this range. In our model, however, the variation in response is too great in this segment of curve (*cf.* Fig. 1, 250 and 500 μg Eac) and testing would need to be prolonged. In the interest of speed, with but slight variation in response, an end point in the second week was chosen, although curves are not parallel in this range. This corresponds to a 50% mortality end point for the 12th day, $LD_{50(12)}$, "lethal dose" referring to the corticoid.

C. *Are certain corticoids less apt to enhance infection from a clinical point of view?* Since glucocorticoid and anti-inflammatory activities, when both assayed in the same host showed divergent ratios in the highly potentiated compounds(16,17), there can be little doubt that different mechanisms of action are involved. Chemical modifications which manipulate this divergence and which should aid in defining biologic mechanisms of action, have been found in the modified progesterone and hydrocortisone series(18,19). The possibility that mechanisms of infection-enhancement and of anti-inflammatory activity might also differ, suggested that certain compounds might have improved therapeutic indices in man when anti-inflammatory effect is desired. Analysis of this problem necessitates comparison of both activities in either hamster or man.

Summary. (1) Chronic latent *Besnoitia* infection in Golden hamsters is highly sensitive to corticoid effects. (2) Fatal relapse from renewed microbial proliferation can be brought about as early as 6 days after initiation of corticoid therapy. (3) By assay which uses as end point the dose producing relapse on 12th day, potency ratios have been determined for 22 modified progestins and corticoids.

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