PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 202-206 (1975)

Glucocorticoid-Induced Protection in Circulatory Shock: Role of Reticuloendothelial System Function[1] (39002)

BURTON M. ALTURA[2]

Departments of Anesthesiology and Physiology, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York 10461 and Department of Physiology, State University of New York, Downstate Medical Center, Brooklyn, New York 11203

Evidence has accumulated that suggests that pharmacologic doses of glucocorticoids may be beneficial in the treatment of circulatory shock (1–4). Several workers, however, question the use of these steroids in the treatment of low-flow states (5–8). In addition, recent findings suggest that prophylactic preshock treatment with these corticosteroids is far superior to postshock treatment (see review by Desmonts and Pocidalo (9)) and that the latter may not result in an increase in overall survival (10–12). However, postshock treatment may be dose-dependent as well as time-dependent (9, 13).

Several mechanisms, (e.g., stabilization of lysosomal membranes, metabolic actions, inotropic actions on the heart, vasodilatation, alpha-adrenergic blockade, immune reactions, curtailment of the constrictor actions of vasoactive hormones), have been advanced to account for this protective effect in shock, but at present there is no agreement as to the precise mechanism(s) involved (4, 8, 9, 14–17). It has recently been demonstrated that pharmacologic doses of glucocorticoids: (i) can accelerate the clearance of endotoxin in rabbits subjected to endotoxin and intestinal ischemia shock (18), and (ii) can aid in the restoration of normal reticuloendothelial system (RES) function in rats subjected to hemorrhagic and intestinal ischemia shock when administered in the postshock period (13). These latter two observations, implicating the RES, could be quite important since considerable evidence has accumulated to indicate that the RES may: (i) represent a homeostatic system serving as a critical common pathway in the pathogenesis of and in host adaptation to circulatory shock syndromes (19–24); and (ii) provide useful diagnostic and prognostic parameters of shock syndromes (21–25).

In view of the foregoing, the present study was designed to determine whether different pharmacologic doses of hydrocortisone sodium succinate (HC) and methylprednisolone sodium succinate (MP): (i) are equieffective as both prophylactic and therapeutic regimens in circulatory shock; (ii) are beneficial as therapy only at a certain time after circulatory shock is induced; and (iii) alter the early RES phagocytic depression characteristic of circulatory shock syndromes (21–28).

Methods. Female rats of the Wistar strain, weighing 150 ± 20 g and anesthetized with im pentobarbital sodium (Nembutal, 30 mg/kg), were used for all of our studies. $LD_{50;60}$ shock procedures were utilized in our experiments. Femoral arteries and veins were cannulated in all animals, including controls. Acute hemorrhage was used to induce circulatory shock. Hemorrhage was induced by gradedly bleeding the anesthetized rats via a cannulated femoral artery over a 20–30 min period to a fixed volume of 3% body weight and withholding the blood for 2 hr (24). After the 2-hr hypotensive period the shed blood was reinfused (intra-arterially) over a 20–30 min period. Two milliliters of Ringer alone or containing different doses of either HC (Solu-Cortef, The Upjohn Co., Kalamazoo, MI) or MP (Solu-Medrol, The Upjohn Co.) was infused iv at a constant rate for 25 min either before hemorrhage (i.e., pretreatment at 2 or 6 hr) or posthemorrhage (i.e., immediately upon return of shed blood or 2 hr after return of

[1] This work was supported by Research Grant Nos. HL-12462 and HL-18002 from the National Heart and Lung Institute, U.S. Public Health Service.

[2] Requests for reprints should be sent to: B. M. Altura, Department of Physiology, Box 31, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York 11203.

shed blood). The cannulas were removed immediately after the Ringer and steroid infusions and the wounds were sutured. The animals were observed for survival for 7 days. Rats that survive such hemorrhage procedures for 7 days are known to live out their entire life spans (24). All hemorrhaged animals were autopsied grossly at death for characteristic signs of shock. Blood pressure was carefully monitored in all of these experiments. The statistical validity of the survival data was assessed by means of the chi-square test.

In other experiments (i.e., untreated shocked animals and steroid-treated shocked animals), RES phagocytic indices (or K values as they are termed) were determined 3 hr posttransfusion; K values were also determined in appropriate paired control animals at similar time intervals. The phagocytic indices were determined from the rate of clearance of colloidal carbon, 4 mg/100 g of body weight, suspended in calf skin gelatin by using a slight modification (29) of the technique of Biozzi and his co-workers (30). Precisely timed blood samples were obtained 2, 4, 8, 12, and 15 min after intravenous carbon injection, hemolyzed in 0.1% sodium carbonate and the carbon concentrations were measured photometrically at 675 nm. Phagocytic indices were calculated:

$$K = (\log C_1 - \log C_2)/(t_2 - t_1)$$

where K is the phagocytic index, C_1 and C_2 are the colloidal carbon concentrations per 100 ml of blood at times t_1 and t_2. The means and standard errors of the means were calculated and statistically analyzed using Student's t test.

Results. Tables I and II show the survival data, as well as the carbon clearance K values, obtained after acute hemorrhage in control and steroid-treated animal. These data indicate several important findings:

TABLE I. INFLUENCE OF PHARMACOLOGIC DOSES OF HYDROCORTISONE ON SURVIVAL AND CARBON CLEARANCE IN RATS SUBJECTED TO ACUTE HEMORRHAGE.[a]

Group and time of steroid administration (± hr)	Dose (mg/kg)	Survivors/ total	Survival (%)	Phagocytic index (K; mean ± SE)
Controls		24/24	100	0.044 ± 0.004 (24)[b]
Hemorrhaged controls	2.0 ml Ringer's	10/26	38	0.011 ± 0.003[c] (12)
Hemorrhage + HC				
−2.0 hr	10	10/18	56	0.014 ± 0.004 (8)
	50	14/18	77[d]	0.025 ± 0.005[d] (8)
	150	17/18	94[d]	0.046 ± 0.008[d] (8)
	300	18/18	100[d]	0.045 ± 0.007[d] (8)
−6.0 hr	10	8/18	44	0.010 ± 0.003 (8)
	50	10/18	56	0.015 ± 0.005 (8)
	150	14/18	77[d]	0.030 ± 0.009[d] (8)
	300	18/18	100[d]	0.047 ± 0.008[d] (8)
0 hr	10	7/18	39	0.009 ± 0.004 (8)
	50	9/18	50	0.010 ± 0.003 (8)
	150	10/18	56	0.013 ± 0.005 (8)
	300	16/18	89[d]	0.024 ± 0.004[d] (8)
	600	12/18	67	0.007 ± 0.003 (8)
+2.0 hr	150	8/18	44	0.009 ± 0.003 (8)
	300	14/18	72[d]	0.020 ± 0.003[d] (8)
	600	7/18	39	0.006 ± 0.002 (8)

[a] All of the hemorrhaged animals were bled 3% body weight. K values were obtained 3 hr after transfusion of shed blood. Survival determined at 7 days.

[b] Number of animals.

[c] Significantly different from control animals ($P < 0.01$).

[d] Significantly different from hemorrhaged controls ($P < 0.03$).

TABLE II. INFLUENCE OF PHARMACOLOGIC DOSES OF METHYLPREDNISOLONE ON SURVIVAL AND CARBON CLEARANCE IN RATS SUBJECTED TO ACUTE HEMORRHAGE[a]

Group and time of steroid administration (± hr)	Dose (mg/kg)	Survivors/ total	Survival (%)	Phagocytic index (K; mean ± SE)
Controls		20/20	100	0.045 ± 0.002 (20)[b]
Hemorrhaged controls	2.0 ml Ringer's	10/25	40	0.010 ± 0.003[c] (12)
Hemorrhage + MP				
−2.0 hr	1	9/18	50	0.011 ± 0.004 (8)
	5	15/18	83[d]	0.030 ± 0.005[d] (8)
	15	18/18	100[d]	0.038 ± 0.006[d] (8)
	30	18/18	100[d]	0.047 ± 0.007[d] (7)
−6.0 hr	1	8/18	44	0.012 ± 0.004 (8)
	5	13/18	72[d]	0.025 ± 0.005[d] (8)
	15	16/18	89[d]	0.032 ± 0.006[d] (8)
	30	18/18	100[d]	0.045 ± 0.005[d] (8)
0 hr	1	9/18	50	0.011 ± 0.003 (8)
	5	10/18	56	0.014 ± 0.005 (8)
	15	12/18	67	0.020 ± 0.006 (8)
	30	15/18	83[d]	0.027 ± 0.005[d] (8)
	60	10/18	56	0.009 ± 0.003 (8)
+2.0 hr	15	7/18	39	0.011 ± 0.005 (8)
	30	13/18	72[d]	0.022 ± 0.004[d] (8)
	60	6/18	33	0.006 ± 0.002 (8)

[a] All of the hemorrhaged animals were bled 3% body weight. K values were obtained 3 hr after transfusion of shed blood. Survival determined at 7 days.

[b] Number of animals.

[c] Significantly different from control animals ($P < 0.01$).

[d] Significantly different from hemorrhaged controls ($P < 0.03$).

(i) massive doses of synthetic glucocorticoid hormones are of significant value in the treatment of rats subjected to acute hemorrhage; (ii) if the steroids are administered prior to induction of shock, lower doses of both hydrocortisone and methylprednisolone can be administered for therapeutic effects than can be given postshock; (iii) MP is approximately 10 times more potent than HC in inducing survival after hemorrhage; (iv) pretreatment of rats with pharmacologic doses of both HC and MP, which promote survival after hemorrhage, either ameliorate or completely prevent the early RES phagocytic depression characteristic of circulatory shock syndromes; (v) administration of rather high doses of both HC (i.e., 300 mg/kg) and MP (i.e., 30 mg/kg) not only can promote survival posthemorrhage but can significantly enhance the depressed RES phagocytic function normally seen in untreated animals subjected to hemorrhage; and (vi) although administration of very high doses of both HC (i.e., 600 mg/kg) and MP (i.e., 60 mg/kg) 2 hr posthemorrhage do not enhance or lower overall survival such high doses may not worsen the already depressed RES phagocytic capacity of rats subjected to hemorrhagic shock.

Discussion. The present experiments support the idea that administration of massive doses of synthetic adrenocorticosteroids may be of considerable value in the treatment of circulatory shock. These experiments thus confirm and extend earlier studies of several workers (1–4, 9–13, 18). Our present findings indicate that, at least for rats subjected to hemorrhage, prophylactic use of these ste-

roids is, however, much more efficacious than is postshock therapy, thus confirming a notion suggested previously by others (9–12). In addition, the present data support the idea that postshock treatment with massive doses of glucocorticoid is probably not only time-dependent but dose-dependent as well (9, 13). For example, (i) only one dose of HC (i.e., 300 mg/kg) and MP (i.e., 30 mg/kg) resulted in enhanced survival when administered posthemorrhage; and (ii) a delay in time of steroid administration (i.e., immediately after transfusion of shed blood vs 2 hr posttransfusion) resulted in less of a therapeutic efficacy.

Considerable evidence has accumulated to suggest that tolerance to various types of experimental shock and trauma may be associated with the functional capacity of the phagocytic elements of the RES (19–29). The present findings indicate that doses of synthetic adrenocorticosteroids which significantly enhance survival after acute hemorrhage either can completely prevent the usual RES phagocytic depression seen early after shock (e.g., if steroid is given prophylactically) from occurring or can ameliorate the RES depression (e.g., if steroid is given postshock). These steroid-induced adaptive RES phagocytic changes, seen only in animals which survive the hemorrhage, are very similar to those seen in animals that: (i) spontaneously survive hemorrhage and other forms of circulatory shock (21–26, 28), and (ii) are tolerant to shock and trauma (22, 25). Enhanced RES function could result in more efficient handling [i.e., inactivation of blood-borne tissue mediators, endotoxins, metabolites and/or other noxious tissue products released in shock (19, 20, 22)]. In fact, one recent report does indeed indicate that therapeutically effective doses of glucocorticoids can actually accelerate the clearance of endotoxin in rabbits subjected to shock (18). Furthermore, lysosomal enzymes are not only degraded (metabolized) by the RES (8, 31) but remain intracellularly in the phagocytic elements of the RES of animals during the reversible phases of circulatory shock (32, 33). Since RE cells are known to take part in many aspects of body metabolism, (e.g., catabolism and anabolism of proteins, carbohydrates, lipids and steroids) (28, 34), it also must be entertained that glucocorticoid-induced reversal of deranged aspects of cellular metabolism (e.g., acidosis, hyperpotassemia, excess lactate) observed by several investigators (4, 9, 14) in patients and animals subjected to shock, could in part be brought about through a stabilization and/or enhancement of RE cell function.

Although some old work has suggested that administration of high doses of glucocorticoid hormones may impair phagocytosis of leukocytes and RE cells from both animals and man (see 34–36 for older references), it must be pointed out that these older studies: (i) were not well controlled; (ii) employed impure glucocorticoids, cortisone or cortisol without its sodium succinate moiety; and (iii) employed repetitive doses of these steroids. Very recent findings of several investigators strongly suggest that even repetitive daily administration (up to 1 g iv) of synthetic HC sodium succinate and MP sodium succinate to patients fails to impair phagocytosis of either latex particles or bacteria by peripheral leukocytes (35–37). The present results could be used to extend the latter concept to fixed RE cells. Even though the present findings, when considered in light of other work discussed above and elsewhere (13, 17) could implicate the RE cells as the pivotal system responsible for steroid protection in shock, it must be emphasized that (i) these experiments are, to our knowledge, limited to a single type of circulatory shock, (ii) they were done in a single species, (iii) it is possible the RES changes may be the end result of the beneficial effects of glucocorticoid administration rather than the cause, (iv) these experiments are limited to clearance of carbon as a test, of RES function (others such as Di Luzio Filkins and co-workers, see references in (34), have demonstrated that colloidal carbon may not always be an adequate measure of other aspects of RES activity), and (v) these experiments do not take into account the possible role opsonins play in the observed RES-steroid carbon clearances (34).

Summary. Experiments with rats indicate that: (i) hydrocortisone sodium succinate

(HC) and methylprednisolone sodium succine (MP) enhance survival after hemorrhage; (ii) MP is approximately 10-times more potent than HC; (iii) both HC and MP are more efficacious if administered prior to hemorrhage; (iv) efficacy of post-shock therapy with both steroids is not only time- but dose-dependent; and (v) HC and MP can ameliorate or completely prevent the early RES phagocytic depression observed in circulatory shock. Overall, these data could be used to suggest that: (i) the RES may play a pivotal role in the beneficial actions of synthetic adrenocorticosteroids in circulatory shock, and (ii) numerical RES phagocytic indices may be diagnostic and prognostic parameters in circulatory shock therapy.

The author wishes to express his thanks to Mrs. J. Hanley and Mr. R. W. Burton for their technical assistance. We are grateful to Marvin Berkowitz of the UpJohn Company for generously providing us with the Solu-Cortef and Solu-Medrol used in these studies.

1. Lillehei, R. C., Longerbeam, J. K., Bloch, J., and Manax, W. G., Ann. Surg. **160,** 682 (1964).
2. Weil, M. H., and Whigham, H., Amer. J. Physiol. **209,** 815 (1965).
3. Motsay, G. J., Alho, A., Jaeger, T., Dietzman, R., and Lillehei, R. C., Fed. Proc., Fed. Amer. Soc. Exp. Biol. Proc. **29,** 1861 (1970).
4. Clermont, H. G., Williams, J. S., and Adams, J. T., Ann. Surg. **179,** 917 (1974).
5. Spink, W. W., and Vick, J., Circ. Res. **9,** 184 (1961).
6. Thomas, C. S., Jr., and Brochman, S. K., Surg. Gynecol. Obstet. **121,** 61 (1968).
7. Replogle, R. L., Kundler, H., Schottenfeld, M., and Spear, S., Ann. Surg. **174,** 126 (1971).
8. Reichgott, M. J., and Melmon, K. L., Med. Clin. North Amer. **57,** 1211 (1973).
9. Desmonts, J. M., and Pocidalo, J. J., La Nouvelle Presse Med. **1,** 2671 (1972).
10. Hakstian, R. W., Hampson, L. G., and Gurd, F. N., Arch. Surg. **83,** 335 (1961).
11. Lefer, A. M., and Martin, J., Amer. J. Physiol. **216,** 314 (1969).
12. Glenn, T. M., and Lefer, A. M., Eur. J. Pharmacol. **13,** 230 (1971).
13. Altura, B. M. and Altura, B. T., J. Pharmacol. Exp. Ther. **190,** 300 (1974).
14. Schumer, W., and Nyhus, L. M., Surg. Clin. North Amer. **49,** 147 (1969).
15. Schumer, W., Erve, P. R., and Obernolte, R. P., Surgery **72,** 119 (1972).
16. Spath, J. A., Jr., Gorczynski, R. J., and Lefer, A. M., Surg. Gynecol. Obstet. **137,** 597 (1973).
17. Altura, B. M., and Altura, B. T., Anesthesiology **41,** 197 (1974).
18. Woodruff, P., Cardis, D., Cuevas, P., Koizumi, S., and Fine, J., Arch. Surg. **107,** 613 (1973).
19. Zweifach, B. W., Brit. J. Anaesth. **30,** 466, (1958).
20. Fine, J., Rutenburg, S., and Schweinberg, F. B., J. Exp. Med. **110,** 547 (1959).
21. Altura, B. M., and Hershey, S. G., Bull. N.Y. Acad. Med. **43,** 259 (1967).
22. Altura, B. M., and Hershey, S. G., Amer. J. Physiol. **215,** 1414 (1968).
23. Altura, B. M., and Hershey, S. G., RES: J. Reticuloendothelial Soc. **10,** 361 (1971).
24. Altura, B. M., and Hershey, S. G., Proc. Soc. Exp. Biol. Med. **139,** 935 (1972).
25. Altura, B. M., and Hershey, S. G., Adv. Exp. Med. Biol. **33,** 545 (1973).
26. Rittenbury, M. S., and Hanback, L. D., J. Trauma **7,** 523 (1967).
27. Lemperle, G., Plast. Reconstr. Surg. **45,** 435 (1970).
28. Schildt, B. E., Acta Chir. Scand. **136,** 359 (1970).
29. Altura, B. M., Hershey, S. G., and Hyman, C., RES: J. Reticuloendothelial Soc. **3,** 57 (1966).
30. Biozzi, G., Benacerraf, B., and Halpern, B. N., Brit. J. Exp. Pathol. **34,** 441 (1953).
31. Glenn, T. M., Lefer, A. M., and Beardsley, A. C., Ann. Surg. **176,** 120 (1972).
32. Bitenski, L., Chayen, J., Cunningham, G. J., and Fine, J., Nature (London) **199,** 493 (1963).
33. Bell, M. L., Herman, A. H., Smith, E. E., Egdahl, R. H., and Rutenburg, A. M., Surg. Forum **21,** 10 (1970).
34. Saba, T. M., Arch. Intern. Med. **126,** 1031 (1970).
35. Chretien, J. H., and Garagusi, V. F., RES: J. Reticuloendothelial Soc. **11,** 358 (1972).
36. Webel, M. L., Ritts, Jr., R. E., Taswell, H. F., Donadio, Jr., J. V., and Woods, J. E., J. Lab. Clin. Med. **83,** 383 (1974).
37. Olds, J. W., Reed, W. P., Eberle, B., and Kisch, A. I., Infection and Immunity **9,** 524 (1974).

Received December 20, 1974. P.S.E.B.M. 1975, Vol. 150.