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Glucocorticoid Induced Protection in Experimental Traumatic Shock (40441)

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Synthetic glucocorticoids (e.g., methylprednisolone, dexamethasone) have been found to improve survival in a variety of types of circulatory shock including hemorrhagic, endotoxic, cardiogenic and bowel ischemia shock (1–4) However, little is known about the effects of these agents in traumatic shock. Although Noble-Collip drum (NCD) trauma has been established as a reproducible model of traumatic shock in the rat since 1942 (5), it has only been during the last few years that intensive efforts have been conducted on the pathophysiologic mechanisms of this interesting type of shock.

It is now established that disruption of lysosomes and the resultant accumulation of acid proteases in the circulating blood (6, 7) is an early feature of Noble-Collip drum trauma. Since glucocorticoids are known to effectively stabilize lysosomal membranes under control (8) as well as shock conditions (9, 10), we studied the effectiveness of methylprednisolone in a standardized model of traumatic shock. In addition to determining the effect of this synthetic glucocorticoid on survival, we assessed its action on the accumulation of cathepsin D, a lysosomal protease, and on myocardial depressant factor (MDF) a toxic factor produced by the ischemic pancreas which depresses myocardial performance (11, 12). These parameters were examined in order to determine the possible mechanism of action of glucocorticoids in traumatic shock.

Methods. Female rats of the Sprague-Dawley strain, weighing 225 ± 15 g, were maintained on Purina lab chow *ad libitum,* in an alternating 12-hr light and dark phase environment. Animals were anesthetized (3–4 hr into the light phase) with sodium pentobarbital 25 mg/kg, i.p. Whole-body trauma was subsequently induced by tumbling each animal 575 revolutions (rev) at 45 rpm in a Noble-Collip drum apparatus previously described (5). This procedure resulted in a well defined picture of traumatic shock characterized by systemic hypotension, splanchnic vascular engorgement and stasis, and the appearance of serosanguinous fluid in the intraperitoneal space. Immediately following trauma, a polyethylene catheter (PE 50) filled with 0.9% NaCl was inserted 4–6 mm into the left carotid artery. A polyethylene tube (PE 205), was also inserted into the trachea to insure a patent airway. The total surgical time was 5–7 min. Heart rate (HR) and mean arterial blood pressure (MABP) were recorded on a Grass model 7 oscillographic recorder using Statham model P23 fluid pressure transducers. In animals receiving steroid, 45 mg/kg of methylprednisolone (Solu-Medrol, Upjohn) was injected (iv) 30 min prior to trauma. Animals given the steroid vehicle (0.9% NaCl containing 0.2% benzyl alcohol) were injected in a similar manner.

Sham-operated control rats were treated in the same way but were not subjected to the Noble-Collip drum trauma. Blood samples (4–5 ml) were drawn from the abdominal aorta at 5 hr post-trauma or when MABP reached 40 mm Hg, thus ending the experiment. Plasmas from the blood samples were assayed for cathepsin D according to the method of Anson (13), and myocardial depressant factor (MDF) according to the method of Barenholz *et al.* (14) with elution of the active region from paper chromatograms according to the technique of Yamada and Pettit (15).

Results. Twenty-eight rats were divided into three experimental groups. Figure 1 illustrates the survival time and final blood pressures of these groups of rats. All sham-operated control rats given methylprednisolone survived the 5-hr observation period. These animals exhibited an initial blood pressure of 118 ± 9 mm Hg and at 5 hr, MABP

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declined to 102 ± 8 mm Hg. These data indicate that methylprednisolone did not adversely influence either blood pressure or survival in nontraumatized animals. In contrast, the rats subjected to drum trauma survived only 2.4 ± 0.4 hr. These rats started with a MABP of 102 ± 5 mm Hg initially and exhibited a final MABP of 45 ± 5 mm Hg representing a significant decrease in pressure ($p < 0.01$). The group of traumatized rats given 45 mg/kg methylprednisolone intravenously, survived significantly longer than the untreated traumatized rats ($p < 0.01$) and exhibited a significantly higher final MABP ($p < 0.01$). In both respects, steroid treated rats were not significantly different from sham-operated control rats. Thus, methylprednisolone significantly improved both survival time and maintained arterial blood pressure in traumatized rats.

Figure 2 summarizes the plasma cathepsin D and MDF activities in the three groups of rats. Rats given methylprednisolone and subjected to a sham trauma protocol exhibited low cathepsin D and MDF activities at the end of the 5-hr observation period. In contrast, untreated traumatized rats developed very high cathepsin D and MDF activities ($p < 0.01$ from sham-operated controls) increasing to fourfold and threefold sham control values respectively. Methylprednisolone treatment significantly retarded the increases

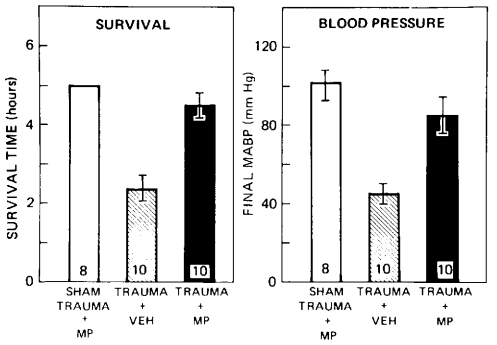

FIG. 1. Survival time and mean arterial blood pressure of the three groups of rats. All values are means, with the number of rats in each group indicated at the bottom of the bars. Brackets indicate standard errors of the means. MP = methylprednisolone, Veh = steroid vehicle. Survival time and MABP are significantly lower ($p < 0.01$) in the trauma + vehicle group than in the other two groups.

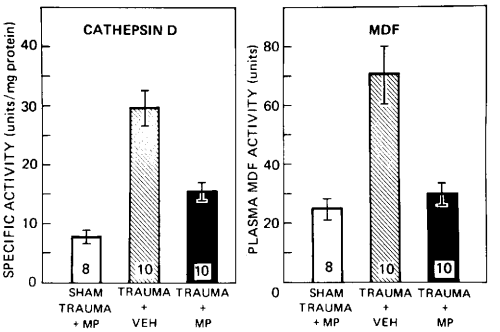

FIG. 2. Plasma cathepsin D and MDF activities of the three groups of rats. All values are means ± SEM. Numbers at the bottom of the bars are the number of rats in each group. Cathepsin D and MDF activities in the trauma + vehicle group are significantly higher ($p < 0.01$) than in the other two groups.

in both substances. Final plasma activities of cathepsin D and MDF were significantly lower than untreated traumatized rats ($p < 0.01$), and the MDF activity was not significantly different from that of sham-operated control rats. These results indicate that methylprednisolone exerts a significant degree of lysosomal stabilization and subsequent prevention of MDF formation in rats subjected to NCD trauma.

Discussion. The results obtained in this study indicate that glucocorticoids can be of significant value in traumatic shock. Using a standardized model of trauma, having a mortality rate of 85%, high doses of methylprednisolone successfully improved the early survival time from 2.4 to 4.5 hr. Earlier investigators also suggested that glucocorticoids may be useful in experimental trauma. Janoff *et al.* (16) employed a similar protocol to the present one and reported that cortisone given to rats prior to trauma conferred some degree of protection on the plasma appearance of the lysosomal hydrolase, acid phosphatase. However, no hemodynamic studies were performed, nor was survival studied. Vander Vennet and Schneewind (17) also studied the effects of steroids in trauma, but did not measure any hemodynamic or biochemical indices of shock. These workers did observe an increased survival rate in rats receiving a variety of glucocorticoids including cortisol, prednisolone, and methylprednisolone. Lahdensuu *et al.* (18) more recently studied the

effects of methylprednisolone on femur fracture induced trauma in the rabbit. They did not observe any change in blood pressure over the six hour posttrauma observation period in animals given the steroid but observed an increased degree of lactacidosis.

We have extended these earlier studies to focus on survival time and some of the important hemodynamic and biochemical variables which could help explain the improved survival time. Our results are consistent with the earlier reports of an improved survival and a protective effect on lysosomes in traumatic shock (17, 18). These results are also in agreement with the general concept that lysosomal disruption and toxic factor formation (e.g., MDF formation) are an important pathophysiologic pathway in all forms of circulatory shock (9, 11). Thus, we postulate that traumatic shock produced by drum trauma alters blood flow distribution and blood volume to produce splanchnic ischemia. We routinely observed splanchnic congestion and the appearance of serous and serosanguinous fluid in the peritoneal space in rats subjected to drum trauma at the time of drawing the final blood sample. These pathological findings were uniformly absent in steroid treated traumatized rats and in sham-operated controls.

The splanchnic ischemia probably leads to lysosomal disruption in the liver and pancreas as observed in other types of shock (10, 19). This accounts for the appearance of cathepsin D in the circulating blood in agreement with the earlier work of Beard and Hampton (7) who reported the progressive increase in serum protease activity in drum trauma in proportion to the degree of trauma. Splanchnic lysosomal disruption, particularly in the ischemic pancreas, leads to the formation of the cardiotoxic peptide, MDF (12). Once MDF is formed, it is transported to the circulation where it depresses the myocardium (19) and impairs hepatic phagocytosis (20). These phenomena are extremely deleterious in circulatory shock and could contribute to the severe mortality observed in our model of traumatic shock. Methylprednisolone, by stabilizing lysosomal membranes, appears to prevent the appearance of lysosomal enzymes, which themselves can damage the microcirculatory endothelium and which also lead to the formation of MDF. We suggest this is a likely mechanism of the protective effects of glucocorticoids in traumatic shock.

Summary. Methylprednisolone (45 mg/kg) given to rats subjected to drum trauma (LD_{85}) significantly increased survival time from 2.4 to 4.5 hr. Coincident with the prolonged survival was a higher mean arterial blood pressure. Methylprednisolone exerted no effect on blood pressure in nontraumatized control rats. Furthermore, traumatic shock resulted in a fourfold increase in circulating cathepsin D activity and a threefold increase in circulating MDF activity. Methylprednisolone significantly ($p < 0.01$) prevented the increase in both substances. The data suggest that methylprednisolone protects in traumatic shock by a mechanism involving lysosomal membrane stabilization rather than by an active hemodynamic action.

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