Modification of Burn Shock Mortality in Mice by an Oxidizing and a Reducing Agent (37155)

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Tissue hypoxia secondary to inadequate perfusion has been assigned an important role in the pathophysiology of shock and, in particular, in the development of so-called irreversibility (1, 2). A shift toward reduction in tissues has been demonstrated in shock, especially after hemorrhage and thermal trauma (3–6). It is known that prolonged oxygen deprivation results in labilization of lysosomal membranes, with release of hydrolytic enzymes (7). It has also been observed that mitchondria from animals in shock are defective in their metabolic function (8). Similar changes in these organelles occur following exposure to reducing agents (9–11). Also, individual proteins and enzymes may undergo changes of conformation and activity, sometimes irreversible, when the oxidation–reduction state of the medium is altered (12–16). These facts suggest that oxidation–reduction, or redox, reactions are involved in the genesis of the subcellular chemical effects of hypoxia. If this were so, it would be expected that reducing agents administered in conjunction with a standardized hypoxic injury should produce more damage than hypoxia alone, while oxidizing agents might, at least partially, protect tissues from the effects of hypoxia. The following experiments were designed as a preliminary test of this hypothesis.

Materials, Methods, and Results. Standardized injury. A burn of two-thirds of the body surface area was produced in NIH female mice (18–20 g weight) by immersion to the axilla in water at 70° for 6 sec.

Oxidizing agent. The vitamin K compound 2-methyl-1,4-naphthaquinol *bis* (disodium phosphate) (MNDP; Synkavite®) was injected intraperitoneally in doses of 5, 7.2, 12.5, or 25 mg/kg in 0.1 ml sterile water. Control animals received 0.1 ml water. These dosages produced no mortality in uninjured mice.

Reducing agent. Cysteine (free base) was injected intraperitoneally in doses of 250, 500, 750, or 1250 mg/kg in 0.3 ml sterile water. Control animals received 0.3 ml water. Again, these dosages produced no mortality in uninjured mice.

Statistical procedures. Mortality was recorded at 6, 18, 24, and 48 hours after injury in all experimental groups. The chi-squared test with continuity correction (17) was used to compare mortality at 48 hr in groups of mice receiving different treatments. The probability $p = 0.05$ was chosen as the level of statistical significance. A difference was judged as significant only when the calculated chi-square equalled or exceeded the tabulated value (1 degree of freedom) at this level.

Experiments. Effect of MNDP and cysteine pretreatment on mortality. MNDP or cysteine was given to animals 20 min before burning. Table I shows that treatment with 5 mg/kg MNDP decreased mortality from 56 to 34% ($p < 0.05$). Higher doses of MNDP were not significantly beneficial or toxic to burned mice. Mortality was significantly higher (76 vs 42%) in animals pretreated with 1250 mg/kg cysteine than in those receiving no cysteine ($p < 0.005$). Lesser doses of cysteine produced no significant decrease or increase in mortality. The apparent decreased

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mortality (28% vs 42%) in mice receiving 250 mg/kg cysteine is not statistically significant.

Effect of MNDP on cysteine toxicity. To test whether the oxidizing agent could counteract the effects on mortality of the reducing agent (or vice versa), mice were given MNDP, with or without a toxic dose of 1250 mg/kg cysteine, 20 min prior to injury. Experiment 1, Table II, shows that, as before, MNDP alone, in the 5 mg/kg dose, reduced mortality at 48 hr from 50 to 24%, while cysteine was toxic and increased mortality from 50 to 72%. In Expt. 2, the toxic dose of cysteine was able to abolish the protective effect of 5 mg/kg MNDP. In Expt. 3, the highest dose tested of MNDP (25 mg/kg) was not, however, able to reverse the effects of the toxic dose of cysteine. Smaller doses of MNDP (not shown in the table) also had no protective effect against cysteine.

Effect of MNDP or cysteine treatment following trauma. Cysteine or MNDP was administered 30 min after burning (Table III). MNDP given after injury did not significantly affect mortality at 48 hr. Cysteine in doses of 500, 750, and 1250 mg/kg increased mortality from 30% to 58% ($p < 0.05$), 94% ($p < 0.005$), and 100% ($p < 0.005$), respectively. These results indicate that burned mice are more sensitive to the toxic effects of cysteine when it is administered after trauma than when it is given beforehand, and that the protective effect of MNDP administered before trauma is not observed when MNDP is injected after injury.

Effect of fluid therapy on cysteine toxicity. Since the results thus far suggest that reducing agents could play a toxic role in the pathogenesis of burn shock, the effect of saline therapy on exogenously administered reducing agent was investigated. In the final experiment, cysteine was given to animals with or without a simultaneous subcutaneous injection of 3 ml saline at 30 min following thermal trauma. Table IV shows that mortality produced by cysteine in all doses was significantly reduced by saline ($p < 0.001$); however, with the higher and toxic doses of cysteine (750 and 1250 mg/kg), saline had a limited, though significant, effect.

TABLE I. The Effect of Cysteine and MNDP, Administered before Trauma, on Shock Mortality of Mice.[a]

Treatment				Cumulative mortality	
H_2O (ml)	Drug	Dose (mg/kg)	No. of mice	24 hr (%)	48 hr (%)
0.3	—	—	50	28	42
0.3	cysteine	250	50	20	28
0.3	cysteine	500	50	22	38
0.3	cysteine	750	50	36	58
0.3	cysteine	1250	50	64	76
0.1	—	—	50	42	56
0.1	MNDP	5	50	22	34
0.1	MNDP	7.2	50	30	40
0.1	MNDP	12.5	50	38	42
0.1	MNDP	25	50	52	64

[a] All treatment was injected ip 20 min before a 2/3 body surface area burn in water at 70° for 6 sec. Food and water were allowed *ad libitum*.

Discussion. Although the redox states of tissues in shock have been little studied, such studies as exist indicate that the oxidation–reduction potential of tissues, as measured with electrodes or by lactic/pyruvate ratios is lowered in hemorrhagic and burn shock (3–6). Our approach was limited, at this time, to the demonstration of a gross effect upon the whole animal with the administration of exogenous oxidizing and reducing agents. Since the results of these experiments show a clearcut effect on mortality, further more refined experiments must be performed in order to relate these findings to endogenous changes in redox state. Because of the paucity of data relating to redox states in the field of shock, it is appropriate to introduce some known facts, which are essential to our argument, from the extensive literature on the role of redox states in radiation injury and oxygen toxicity.

In simple chemical systems, the equilibrium redox potential, E_h, may be measured with electrodes. This method is not always reliable in tissues (5, 18). Tissue redox state, or steady-state E_h, is commonly estimated from

TABLE II. The Effect of MNDP on the Toxicity of Cysteine in Burn Shock.[a]

Experiment	Treatment H_2O (ml)	+ MNDP (mg/kg)	+ cysteine (mg/kg)	No. of mice	Cumulative mortality 24 hr (%)	48h r (%)
1	0.4	—	—	30	33	50
	0.4	—	1250	39	55	72
	0.4	5	—	29	10	24
2	0.4	—	1250	39	55	72
	0.4	5	—	29	10	24
	0.4	5	1250	30	67	80
3	0.4	—	1250	39	55	72
	0.4	25	—	27	59	59
	0.4	25	1250	30	67	87

[a] All treatment was injected ip 20 min before a 2/3 body surface area burn in water at 70° for 6 sec. Animals were allowed food and water *ad libitum*.

the concentrations of oxidized and reduced forms of any of several chemical species. The lactate–pyruvate system (19–21), pyridine and flavin nucleotides (22, 23), and various indicator dyes (24) have been used for this purpose. It is established, based on measurements of steady-state E_h by the above mentioned techniques, that there is a normal range of tissue E_h, and a gradient of decreasing E_h from extracellular fluid to cytosol to mitochondria (19, 25).

Tissue E_h is the resultant of the interactions of many oxidation–reduction systems; an operational definition of biochemical oxidizing and reducing agents can be given by their respective tendencies to raise and lower E_h. While oxygen is the most important oxidizing agent in the body economy, a knowledge of P_{O_2} alone is insufficient to specify the redox state without information on the "chemical anatomy" of nonvolatile oxidizing and reducing substances in tissue (26). A familiar, analogous situation exists in acid–base chemistry, *e.g.*, it is impossible to determine pH from P_{CO_2} alone.

A variety of sulfhydryl (SH) compounds, including cysteine, lower E_h, and increase the tissue sulfhydryl/disulfide ratio (SH/SS), which varies inversely with E_h. Oxygen deprivation has the same effects (27–29). Both SH compounds and hypoxia protect tissues from radiation injury. It is thus proper to assert that cysteine, whatever other action it may have, behaves as a reducing agent in tissue.

Elevated oxygen tensions raise E_h, decrease SH/SS, and potentiate radiation damage (30, 31). MNDP is also a radiosensitizer (32), and lowers tissue SH (33); the acute toxicity of MNDP is antagonized by cysteine (34). However, MNDP has been reported to lower tissue E_h as measured with electrodes (28, 32); this raises suspicions that the compound may not always act as

TABLE III. The Effect of Cysteine and MNDP, Administered after Trauma, on Shock Mortality of Mice.[a]

Treatment H_2O (ml)	Drug	Dose (mg/kg)	No. of mice	Cumulative mortality 24 hr (%)	48 hr (%)
0.3	—	—	50	26	30
0.3	cysteine	250	50	30	36
0.3	cysteine	500	50	46	58
0.3	cysteine	750	50	92	94
0.3	cysteine	1250	50	100	100
0.1	—	—	50	50	50
0.1	MNDP	5	50	44	46
0.1	MNDP	7.2	50	60	60
0.1	MNDP	12.5	50	50	50
0.1	MNDP	25	50	50	62

[a] All treatment was injected ip 30 min after a 2/3 body surface area burn in water at 70° for 6 sec. Food and water were allowed *ad libitum*.

TABLE IV. Protective Effect of Saline when Cysteine was Given Postburn.[a]

H_2O (ml)	Drug	Dose (mg/kg)	No. of mice	Cumulative mortality without saline therapy 24 hr (%)	Cumulative mortality without saline therapy 48 hr (%)	No. of mice	Cumulative mortality with saline therapy (3 ml sc 30 min after burn) 24 hr (%)	Cumulative mortality with saline therapy (3 ml sc 30 min after burn) 48 hr (%)
0.3	cysteine	250	26	62	65	63	0	3
0.3	cysteine	500	27	59	78	64	5	9
0.3	cysteine	750	30	90	93	70	20	27
0.3	cysteine	1250	30	100	100	70	57	63
0.3	—	—	30	50	60	70	1	4

[a] All mice received a 2/3 body surface area burn at 70° for 6 sec. Cysteine was given ip and saline sc 30 min after injury. Mice were not allowed food or water.

an oxidizing agent.

Our experiments demonstrate that high doses of cysteine, a reducing agent, are toxic to burned animals when given before or after injury. MNDP, an oxidizing agent, protects burned mice at moderately high doses, but only when given before injury.[2] These results indicate that agents which can influence the redox state can also affect mortality resulting from burn injury. Further substantiation comes from the findings that: (1) the reducing agent was able to block the protective effects of the oxidizing agent; and (2) when saline was given to burned mice to correct the sodium deficiency, to improve the circulation, and thereby to increase oxygenation of the tissues, the toxic effects of cysteine were markedly diminished. On the other hand, no evidence could be obtained to demonstrate a reversal of the toxic effect of cysteine by MNDP. This may have been due to the dosages utilized, the schedule or mode of drug administration, or the kinds of drugs used. As mentioned above, the conflicting results with various methods of measurement suggests that MNDP may not always behave as an oxidizing agent; other compounds might be more effective.

Our findings are similar to those of Einheber, Wren, and Klobukowski (35), who administered various radioprotective sulfhydryl reducing agents to mice before the animals were subjected to drum or tourniquet shock. These authors' experiments were carried out in the hope that the agents used might protect against other types of trauma as well as against radiation injury. On the contrary, the treated groups exhibited higher mortality; the toxic effects of the SH compounds were, however, reversed by saline treatment.

These results support the hypothesis that the overall redox balance is a determinant of the severity of hypoxic damage. Lowering E_h with nonvolatile reducing agents should increase, and raising E_h with nonvolatile oxidizing agents should decrease, the lethality of a standardized hypoxic injury. According to this hypothesis, alterations in tissue E_h associated with drug administration or coexistent metabolic disease could profoundly affect the individual's response to trauma. Deliberate metabolic modification of redox states might provide a new biochemical pharmacologic approach to the treatment of hypoxic injury. For these reasons, we feel that tissue redox states in shock deserve fuller investigation.

Summary. A reducing agent, cysteine, or an oxidizing agent, 2-methyl-1,4-naphthaquinol *bis* (disodium phosphate) (MNDP), was given to mice subjected to burn shock by scalding, to test the theory that metabolic modification of tissue redox states should affect the response to hypoxic injury. Cysteine increased mortality in burned mice when given before or after thermal injury. MNDP decreased mortality when administered before, but not after burning. The toxicity of

[2] The protective effect of MNDP is not due to its sodium content, since (1) the amount of sodium in the effective dose is miniscule and (2) increased doses of drug (and, therefore, sodium) do not protect the animals.

cysteine was reduced significantly by administration of saline. This effect is presumably attributable to improved tissue perfusion and oxygenation. These preliminary results suggest that the administration of oxidizing or reducing agents can influence shock mortality after thermal injury, and that metabolic modification of tissue redox states may provide a new approach to the therapy of shock.

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