The Influence of Mannitol on Myoglobinuric Acute Renal Failure: Functional, Biochemical, and Morphological Assessments

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ABSTRACT

This study was undertaken to explore the protective influence of mannitol against the glycerol model of myoglobinuric acute renal failure. Three hypotheses were tested: (1) mannitol confers cytoprotection by acutely blunting renal hypoperfusion, thereby improving tubular cell energetics; (2) as an hydroxyl radical (OH•) scavenger, mannitol mitigates Fe-driven lipid peroxidation and, hence, decreases tubular cell necrosis; and (3) mannitol prevents intrarenal heme pigment trapping, decreasing cast formation. Rats were injected with 50% glycerol (10 mL/kg im), followed immediately by an iv mannitol (1.25 ml/100 g over 1 h) or sham infusion. Mannitol induced a brisk diuresis (~5.7 mL/2 h; ~35 mg of heme protein excreted), whereas glycerol controls were anuric. Mannitol did not significantly increase postglycerol RBF (2.8 ml/min), and it paradoxically worsened cellular energetics, halving cortical ATP concentrations at 1 h. However, this adverse effect on ATP was transient, correlating with active diuresis. Glycerol did not induce convincing in vivo lipid peroxidation (malondialdehyde; conjugated diene assay), and mannitol did not block Fe-driven in vitro lipid peroxidation of isolated brush border membrane vesicles. Na benzoate, an OH• scavenger, conferred no in vivo or in vitro protection. However, Na2SO4, not an OH• scavenger, reproduced the diuretic and in vivo protective effects of mannitol. Purified myoglobin infusion (35 mg) largely negated the beneficial action of mannitol. It was concluded that mannitol confers functional but not cytoprotection against the glycerol acute renal failure model, it acutely worsens renal bioenergetics, and its protective influence is probably due to a diuretic, not an antioxidant, effect.

Key Words: Glycerol, iron, hydroxyl radical, ATP, malondialdehyde

Mannitol is widely prescribed for the early treatment of crush syndrome/myoglobinuric acute renal failure (Mgb-ARF). This is based on clinical (1–3) and experimental (4,5) data which indicate a protective influence if it is administered during the early phase of renal damage. However, the mechanism by which mannitol confers protection remains poorly defined (6). Three possible explanations are frequently advanced. First, through its vasodilatory action (6,7), mannitol might blunt the renal vasoconstriction that typically accompanies the initiation phase of Mgb-ARF (8). Because Mgb and renal ischemia cause synergistic tubular damage (e.g., see references 9–13), an abrogation of renal vasoconstriction could produce cytoprotection. Second, recent evidence suggests that heme Fe can drive hydroxyl radical (OH•) formation within the kidney via the Fenton/Haber Weiss reactions (14,15); because mannitol can scavenge OH• (16), its protective influence could be mediated by an antioxidant effect. Third, acting as an osmotic diuretic, mannitol might decrease heme pigment cast formation and tubular obstruction, thereby lessening the severity of ARF. The purpose of the investigation presented here was to discriminate among these three possibilities. To this end, the glycerol model of Mgb-ARF has been studied because it causes marked renal vasoconstriction (8), because it has been reported to induce oxidant tubular damage (14,15), and because profound renal heme pigment accumulation results. Because of these characteristics, it is the most widely used model for studying Mgb-ARF (1).

METHODS

Effects of Therapy on the Severity of Glycerol-Induced ARF

Female Sprague-Dawley rats (200 to 250 g; Bantin Kingman, Fremont, CA), maintained under standard...
laboratory conditions with free food and water access, were used for all experiments. They were lightly anesthetized with pentobarbital (30 mg/kg) and injected im with 50% glycerol (10 mL/kg) in equally divided doses into each hind limb. The rats were divided into five experimental groups.

Group 1—Glycerol Alone (N = 12). These rats were subjected to glycerol injection with a sham jugular vein infusion.

Group 2—Glycerol Plus Mannitol Treatment (N = 7). These rats underwent glycerol injection, and then a 1-h infusion of 25% mannitol was started. A total of 1.25 mL/100 g body wt was administered iv as a constant infusion, beginning with a 0.5-mL prime.

Group 3—Glycerol Plus Na₂SO₄ Treatment (N = 6). These rats were treated in an identical fashion to those in Group 2, but equiosmolar Na₂SO₄ was substituted for the 25% mannitol.

Group 4—Glycerol Plus Na Benzoate Treatment (N = 5). To assess whether an OH⁺ scavenger other than mannitol would protect against glycerol-induced ARF, five rats received Na benzoate (150 mg/kg ip), followed by glycerol injection. Eight hours later, the Na benzoate dose was repeated. Of note, this benzoate protocol (15) has been previously shown to protect against a different form of glycerol-induced ARF (see Discussion).

Group 5—Exogenous Mgb Infusion (N = 6). To ascertain whether a mannitol diuresis confers protection via its ability to increase Mgb/hemoglobin excretion, six rats were treated in an identical fashion to Group 2, but, 1 h after the completion of the mannitol infusion (the end of the diuretic period), each rat received 35 mg of horse skeletal muscle Mgb (Sigma Chemical Co., St. Louis, MO; dissolved in 1 mL of 5% dextrose/water, infused over 30 min) through the jugular venous catheter. This dose was chosen because it was the approximate amount of heme protein excreted during mannitol infusion (see Results). Thus, the glycerol plus mannitol protocol was performed in an attempt to negate the influence of the diuretic on heme protein excretion.

Urine output was determined for the first 2 h after glycerol injection (urine passed spontaneously or with slight suprapubic pressure). After each hour, an equal volume of 0.45% NaCl was administered ip to approximately replace the Na losses. The total amount of myo/hemoglobin excreted was estimated by determining the total urinary protein concentration (BCA protein assay; Pierce Chemical Co., Rockford, IL), because it was assumed that minimal non-heme protein would be excreted. Two hours after glycerol injection, the rats were allowed to recover from anesthesia; free food and water access were again provided. Twenty-four hours after glycerol injection, the rats were reanesthetized and killed by aortic puncture. The plasma was used for creatinine determination as an index of the severity of ARF. Selected left kidneys were fixed in methyl Carnoy’s solution. Six kidneys from Groups 1 and 2 were processed for light microscopy (paraffin embedded; 6-μm sections; stained with hematoxylin and eosin) to gauge the effect of mannitol on the severity of histological damage.

Mannitol Effects on Cellular Energetics

RBF. Ten rats were injected with glycerol, half with and half without mannitol infusion. Thirty minutes later, the period of maximal diuresis, the left renal artery was exposed through a midline abdominal incision. An electromagnetic flow probe was positioned around the renal artery, and, after approximately 10 min of equilibration time, RBF was determined for 20 min as previously described (17).

Adenine Nucleotide Assay. To determine the effect of mannitol on cellular energetics, renal cortical adenine nucleotides were quantified in the presence and absence of a mannitol diuresis. Eleven rats were injected with glycerol, six without and five with mannitol infusion (treated as per Groups 1 and 2). After 60 min (a time of brisk diuresis in the mannitol group), the left kidneys were snap-frozen in liquid nitrogen (9) and cortical tissues were assayed for ATP, ADP, and AMP by HPLC (18), as previously performed by this laboratory (9).

To assess whether mannitol impacts on renal adenine nucleotide levels after the cessation of the diuresis, 14 rats were injected with glycerol, 9 without and 5 with mannitol infusion. Two hours after glycerol injection (1 h after the cessation of mannitol infusion, corresponding to the end of diuresis), the left kidneys were harvested and assayed for ATP, ADP, and AMP as described above.

Assessment of Oxidant Tissue Damage

Whole Renal Tissues. To determine whether glycerol caused oxidant renal damage, selected Group 1 rats (glycerol injection alone) had their kidneys excised 24 h postinjection, and, instead of being fixed for histology, their cortices were dissected and assayed for conjugated dienes (N = 4) and/or malondialdehyde (MDA; N = 5) as indices of lipid peroxidation. Conjugated dienes were measured according to the method of Recknagel and Glende (19), results being expressed as absorbance units at 233 nm/mg of tissue protein (19). Regarding the MDA assay, heme proteins are known to cause colorimetric interference, because they, like MDA, absorb at 532 nm. This results in an overestimate of MDA concentrations (20). Because of substantial renal heme protein accumulation in the glycerol ARF model, three different methods of expressing the MDA data were employed: (1) uncorrected values (A at 532 nm); (2)
the 532 – 510 nm correction method of Uchiyama and Mihara (21) as previously employed by this laboratory (20); and (3) subtraction of the absorbance value of the tissue homogenates at 532 nm after performing the MDA assay in the absence of the active reagent (thiobarbituric acid). Renal cortices from five normal rats served as controls for the conjugated diene and MDA assays. All samples were run in duplicate.

BBMV. To further assess whether the glycerol model induces lipid peroxidation at a time of active renal injury, three rats were injected with glycerol and, 2 h later, the kidneys were excised. Brush border membrane vesicles (BBMV) were prepared by a modification of the method of Molitoris and Simon (22). Briefly, the cortices were homogenized and the BBMV was recovered by differential centrifugation and magnesium precipitation in a buffer containing 5 mM EGTA and 300 mM mannitol (22). The final centrifugation step replaces EGTA and mannitol with normal saline. This procedure uses sufficient washings to remove all nonbound Mgb from the BBMV preparation (insufficient Mgb remains to impact on the MDA reaction by colorimetric interference). BBMV were assayed immediately for MDA by the 532 – 510 nm subtraction technique. (The small quantities of BBMV obtained precluded performance of all three assay methods on a given preparation.) Two separate BBMV samples were assayed per rat (total N = 6). The results were compared with those found in BBMV harvested from six normal rats (12 determinations).

To validate that the MDA assay when applied to BBMV detects Fe-mediated lipid peroxidation, samples of BBMV obtained from six normal rat kidneys (12 samples) were incubated at 37°C for 30 min with 1 mM FeCl₃–1 mM FeCl₃, the latter was solubilized with 5 mM ADP (16), and the MDA assay was then repeated. To assess whether mannitol or Na benzoate blunts this reaction, these compounds (100 mM) were added to six of the BBMV preparations just before the Fe challenge and, after 30 min, these samples were also assayed for MDA to assess whether lipid peroxidation was prevented.

Calculation and Statistics

All values are presented as means ± 1 SE. Statistical analyses are presented below.

RESULTS

Assessments of the Severity of ARF

Functional Assessments. Glycerol caused severe ARF in all rats, with the 24-h creatinine concentrations ranging from 2.4 to 3.2 mg/dL (95% confidence band, 2.3 to 3.3 mg/dL; Figure 1). Mannitol conferred protection in each rat so tested (all values below the confidence band), and the mean 24-h creatinine for the group was 1.5 ± 0.2 mg/dL. Comparable protection was afforded by equiosmolar Na₂SO₄ (Figure 1). Na benzoate had no beneficial effect; in fact, two of these rats developed greater creatinine increments than the glycerol only group. For reference, a normal rat plasma creatinine is 0.3 to 0.5 mg/dL. Thus, mannitol and Na₂SO₄ induced only incomplete protection.

Morphology Assessments. The Group 1 (glycerol alone) rats demonstrated striking heme pigment cast formation in both cortex and outer medullary stripe, involving approximately 50% of all tubular segments (Figure 2a). In contrast, Group 2 (mannitol) rats showed casts in only ~10% of tubular segments (Figure 2b) and those were predominantly seen in the outer medullary stripe. A semiquantitative scoring of this finding (1+ to 5+ or 10 to 50% of segment involvement) revealed a statistically significant dif-

Figure 1. Plasma creatinines (mg/dL) 24 h after the glycerol injection protocols. The shaded area represents the 95% confidence band for the rats treated only with glycerol (Group 1, controls). All mannitol- and Na₂SO₄-treated rats demonstrated functional protection. Mgb infusion negated that protection in four of six rats so tested. Na benzoate conferred no protection with two rats developing greater creatinine increments than the glycerol only group. For reference, a normal rat plasma creatinine is 0.3 to 0.5 mg/dL. Thus, mannitol and Na₂SO₄ induced only incomplete protection.
Figure 2. Renal histology 24 h after glycerol injection with and without mannitol treatment. The extent of heme protein cast formation was far greater in the Group 1 (glycerol alone; panel a) than the Group 2 (glycerol plus mannitol; panel b) rats (cortical region depicted). However, both groups had extensive and comparable degrees of tubular necrosis (glycerol alone, panel c; glycerol plus mannitol, panel d) (outer medullary stripe depicted).

ference between the two groups ($P < 0.01$; Wilcoxon rank sum test). Proximal tubular cells appeared heavily heme pigment laden (eosinophilic appearance), particularly in Group 1. Proximal tubular cell necrosis was observed in the cortex, medullary rays, and outer medullary stripe. Despite the fact that the mannitol-treated group demonstrated functional protection, the extent of necrosis was just as striking in the mannitol as in the untreated glycerol group. This assessment was made by grading necrosis with a semiquantitative scale of 1+ to 5+ or 10 to 50% of proximal tubular segment involvement (mannitol, 2.5 ± 0.3; controls, 2.2 ± 0.2; not significant, Figure 2c and d).

Assessments of Cellular Energetics

RBF was markedly reduced in the untreated glycerol rats (2.8 ± 0.3 mL/min; normal RBF, ~5 mL/min; [17]). Mannitol exerted no significant renal vasodilatory effect (3.4 ± 0.4 mL/min; not significant versus glycerol alone).

Adenine nucleotide analysis indicated that a mannitol diuresis exacerbated, rather than improved, cellular energetics. At 1 h after glycerol injection, a time of an active diuresis, ATP was approximately 50% lower in the mannitol versus the untreated glycerol group (Table 1; top). The mannitol-induced ATP loss was due to its shift to ADP and AMP, rather than to a loss of total adenine nucleotide (TAN) content. This caused significant increments in ADP and AMP concentrations compared with the untreated glycerol rats. At 2 h after glycerol injection, a time by which the mannitol diuresis had ceased, significant differences in the adenine nucleotide profiles were no longer apparent between the two groups (Table 1; bottom).

### Table 1. Effect of mannitol on renal adenine nucleotide content

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<tr>
<th></th>
<th>N</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>TAN</th>
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<td></td>
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<td></td>
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<td>&lt;0.001</td>
<td>NS</td>
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<tr>
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<tr>
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<td>NS</td>
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* Adenine nucleotide concentrations (nanomoles per milligram dry weight) in postglycerol-injected rats with and without mannitol infusion. The values in parentheses represent 1 SEM. TAN, total adenine nucleotide or the sum of ATP plus ADP plus AMP. As a point of reference, normal values for this laboratory are: ATP, 8.9 ± 0.3; ADP, 3.3 ± 0.1; AMP, 0.7 ± 0.05; TAN, 12.9 ± 0.4 nmol/mg dry wt (9).
Assessments of Oxidant Injury

Whole Cortical Tissues. The 24-h postglycerol-injected rats (Group 1) had no significant increment in cortical conjugated diene concentrations compared with values obtained from normal kidneys (0.17 ± 0.01 versus 0.16 ± 0.01 absorbance per milligram of protein respectively). MDA values were elevated in the 24-h Group 1 rats compared with normal kidneys, when assessed by uncorrected 532-nm absorbance (825 ± 37 versus 614 ± 37 nmol/g dry wt; \( P < 0.01 \)). (This result is analogous to those previously reported in the literature \([14,15]\).) However, when these values were corrected for the color difference in the tissue homogenates (Figure 3), a glycerol-induced MDA elevation was no longer apparent. By using the 532 - 510 nm correction method, the MDA was paradoxically lower in the glycerol group (150 ± 62 versus 446 ± 35; \( P < 0.001 \)). By using the tissue blank method (reaction run in the absence of thiobarbituric acid) the glycerol group still had slightly lower MDA concentrations than did the control tissues (427 ± 47 versus 586 ± 33; \( P < 0.05 \)).

BBMV. The 95% confidence band for MDA obtained on normal BBMV was 0 to 1.75 nmol/mg of protein. Each BBMV sample obtained from rats 2 h postglycerol injection fell within this normal range (Figure 4). The adequacy of the employed MDA method (532-510-nm subtraction) for assessing lipid peroxidation in this system was indicated by 5 to 30-fold MDA increments when exogenous Fe was added to normal BBMV preparations (Figure 4). However, when that challenge was repeated in the presence of mannitol or Na benzoate, no blunting of the MDA increase resulted (Figure 4).

DISCUSSION

Although mannitol is widely used to treat patients with insipient Mgb-ARF, the reason for its efficacy has not been resolved \([6]\). Thus, we have studied the influence of mannitol on three purported determinants of the glycerol ARF model to gain new mechanistic insights into its therapeutic efficacy. The glycerol model was chosen rather than heme pigment infusion (e.g., see references 23 and 24) because the former produces muscle necrosis and intense renal vasoconstriction, possibly better simulating clinical rhabdomyolysis/crush syndrome states \([1]\). Most studies with this model have used 12 to 24 h of dehydration/starvation before glycerol injection because the resulting 5 to 10% body wt loss/volume depletion predisposes to heme pigment injury \([8]\). However, we chose to avoid this variable because our 10-mL/kg glycerol injection protocol routinely induced severe ARF in our rats. Thus, we felt that prior volume depletion both was unnecessary and would compound data interpretation. By this approach, the protective influence of mannitol against Mgb-ARF was confirmed because the mean 24-h plasma creatinine concentration was decreased from 2.8 to 1.5 mg/dL. However, it should be noted that this protection was incomplete because a normal rat serum creatinine in this laboratory is 0.3 to 0.5 mg/dL.

By using this protocol, we first tested the hypothe-
sis that mannitol confers protection by blocking the renal hypoperfusion typical of the postglycerol injection/Mgβ period (8). Thus, RBF was measured from 30 to 60 min after glycerol injection with and without mannitol treatment. The glycerol-treated rats demonstrated an approximate 50% reduction in RBF (2.8 mL/min) compared with normal values (~5 mL/min; [17]). Surprisingly, mannitol, normally a potent renal vasodilator (6), failed to induce a significant increment in renal perfusion. The reason for this result is unknown. It cannot be ascribed to the presence of damaged renal parenchyma because this laboratory has documented previously that mannitol has a marked vasodilatory action in postischemic ARF (7). Of note, heme proteins scavenge nitric oxide, a potent renal vasodilator (25), and glycerol causes an approximate 70% reduction in cardiac output (8), possibly due, in part, to a direct myocardial effect (fructose- and glycerophosphate formation/ATP depletion) (26,27). If the normally marked renal vasodilatory action of mannitol were either nitric oxide or cardiac output dependent, these two considerations could explain the absence of significant RBF enhancement in the present experiments.

Because renal hypoperfusion and renal ischemia are not synonymous, we next sought to determine the overall impact of mannitol on cellular energetics by analyzing cortical adenine nucleotide pools. These assessments were performed at 1 and 2 h post-glycerol injection, corresponding to periods of active diuresis and antidiuresis, respectively. During active diuresis, mannitol worsened, rather than improved, cortical energetics, decreasing ATP concentrations from approximately 5 to 2.6 nmol/mg of tissue dry wt. There are two possible explanations for this finding. First, although a natriuresis normally decreases ATP consumption by decreasing Na transport, were mannitol to increase GFR (e.g., by decreasing casts) and, hence, increase the filtered Na load, a net increment in tubular Na reabsorption (and ATP consumption) could result. We could not directly test this hypothesis because the Group 1 rats remained anuric during the postglycerol injection period. However, micro-puncture experiments have demonstrated that mannitol does, in fact, increase GFR immediately after glycerol administration (5). Furthermore, that the Group 2 and Group 1 rats were polyuric and anuric, respectively, implies a marked difference in GFR. Second, mannitol is known to increase the energy cost of each microequivalent of Na transported (28). Thus, independent of changes in GFR and the filtered Na load, mannitol could still increase tubular metabolic work and, hence, decrease ATP concentrations.

The pathophysiological significance of the mannitol-induced energy depletion can only be surmised. However, it is tempting to postulate that it had an adverse impact. Normally, decreased renal myoglobin accumulation decreases the extent of tubular necrosis (23). However, in the experiments presented here, mannitol did not mitigate tubular necrosis despite a marked decrement in heme pigment accumulation (Figure 2). This suggests that mannitol had a counterbalancing adverse effect on tubular cell integrity, such as an exacerbation of energy depletion. That only cast formation, not cell necrosis, was reduced may explain the incomplete protective effect of mannitol. It should be noted that the impact of mannitol on cellular energetics was a transitory one because, by the end of the diuretic period (2 h), no difference in ATP concentrations existed between the groups. Thus, the adverse influence of mannitol on cellular energetics appears to have been confined, and mechanistically linked, to an active diuresis.

The second hypothesis tested in this study was that mannitol, an OH* scavenger, confers protection by decreasing Fe-driven oxidant tissue damage. The first approach taken was to determine whether glycerol-induced renal heme pigment accumulation induced lipid peroxidation and, if so, to ascertain whether mannitol blunts this reaction. The 24-h tissue samples indicated an MDA increment over normal values by the uncorrected 532-nm analysis, as previously reported in the literature (14,15). However, when these values were adjusted for the heme pigment interference (by using two different methods), this increment was no longer apparent. If anything, the MDA were lower in the glycerol group. We next sought to document oxidant damage in the 24-h postglycerol kidneys by assessing cortical conjugated diene content—a procedure that is not influenced by heme pigment contamination. However, no increment was found. The third approach taken was to assess whether lipid peroxidation could be demonstrated in isolated brush border membranes (BBM) during the initiation phase of renal injury (2 h post-glycerol injection). This subcellular preparation was chosen because the BBM is an early target of Mgβ damage (23) and because, during their isolation, nonmembrane-bound Mgβ (e.g., within lumina) is removed from the vesicle preparation. These assessments were conducted using the MDA assay because the BBM contained insufficient heme pigment to induce substantial assay interference. By this approach, no MDA increment could be found. To validate the MDA assay, normal BBMV were challenged with Fe ex vivo and 5- to 30-fold MDA increments resulted (Figure 4). Thus, these data clearly indicate that the BBM can undergo Fe-triggered lipid peroxidation and that this can be detected by the MDA technique. Furthermore, the adequacy of this assay for detecting biologically relevant degrees of lipid peroxidation has previously been demonstrated in this laboratory with isolated proximal tubular cell preparations (20).
Because the above in vitro and in vivo experiments did not provide convincing evidence of lipid peroxidation. It was not possible to directly determine whether mannitol conferred protection by decreasing oxidant tissue stress. However, we reasoned that if a second OH scavenger, Na benzoate (15,16), mimicked, at least in part, the protective influence of mannitol, this would strongly suggest that the latter had exerted a significant antioxidant action. However, benzoate failed to confer protection in our model. To more directly test whether mannitol blocks Fe-mediated lipid peroxidation, either mannitol or benzoate was added to the ex vivo BBMV/Fe reaction and both failed to blunt the MDA increment. Thus, this ex vivo experiment supports the conclusions from the whole animal data that the protective effect of mannitol is not mediated by a decrease in lipid peroxidation. That mannitol and benzoate failed to blunt the MDA increment in the BBMV/Fe reaction suggests that the documented in vitro lipid peroxidation occurred via a non-OH-dependent reaction.

These results seemingly contradict a previous report by Shah and Walker, which convincingly demonstrated that the OH scavengers (e.g., Na benzoate) can protect against the glycerol ARF model (15). However, it is important to recognize that several methodological differences exist between this study and that one: Walker and Shah used male rats, ether anesthesia, 24 h of prior starvation/dehydration, and a lower glycerol dose (8 mL/kg). Each of these differences could potentially explain our discrepant findings. For example, ether can induce peroxide formation (29), potentially predisposing to oxidant stress. Thus, the absence of lipid peroxidation in our study and our failure to document benzoate-mediated protection only indicate that Fe-driven OH formation is not a constant, or necessary, mediator of Mgb renal injury. This conclusion is supported by results of two previous experimental studies of heme pigment-induced ARF, which employed i.v. hemoglobin (24) or Mgb infusion (23); in those studies, as in this one, renal lipid peroxidation did not result and OH scavengers conferred no protection. However, these past and present results by no means exclude the possibility that, under different experimental conditions, heme Fe might induce in vivo oxidant tissue stress.

The final goal of this study was to test the hypothesis that mannitol induces protection by preventing renal heme pigment accumulation/cast formation due to its diuretic effect. To evaluate this possibility, rats were infused with equimolar NaSO4 to assess whether comparable protection would result. NaSO4 reproduced the urine and heme protein excretion rates of mannitol, and it had the same protective influence. Because NaSO4 is not an antioxidant, these results strongly indicate that the beneficial influence of mannitol is due to enhanced heme protein washout. Two other pieces of information support this conclusion: (1) the 24-h histological assessments demonstrated strikingly lower renal heme pigment accumulation in the mannitol versus the untreated glycerol rats; and (2) when mannitol-treated rats were posttreated with iv Mgb, its protective influence was mitigated. It is tempting to postulate that the ability of mannitol to prevent heme protein cast formation/tubular obstruction explains the improvement in renal function, particularly because it did not lessen the extent of tubular necrosis. Of note, cast formation has been suggested to be an important pathophysiological mechanism for the reduction of GFR in experimental Mgb-ARF (1). However, decreased cast formation could also be a result, as well as a cause, of the functional protection because a higher GFR could secondarily enhance cast excretion.

In conclusion, this study demonstrates that: (1) mannitol confers functional but not cytoprotection against the glycerol model of Mgb-ARF; (2) when administered in a clinically relevant dose, it does not dramatically alter RBF in the early postglycerol injection period; (3) it worsens, rather than improves, cellular energetics despite its natriuretic effect; (4) although it is an OH scavenger, this action does not appear to contribute to its in vivo protective influence: (5) it does not convincingly block Fe-mediated lipid peroxidation of isolated BBM; and (6) its in vivo protective influence can be explained solely by its diuretic action which prevents intrarenal heme protein trapping. Finally, it is important to emphasize that although glycerol injection reproduces many salient features of clinical rhabdomyolysis-induced ARF, it also has independent metabolic effects (e.g., P, trapping/ATP depletion/glycerophosphate formation) (25,26). Thus, conclusions based upon this model should be extrapolated to the clinical arena with caution.

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