Synergistic renal protection by combining alkaline-diureis with lipid peroxidation inhibitors in rhabdomyolysis: possible interaction between oxidant and non-oxidant mechanisms

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Abstract

Background and purpose. Heme-proteins, besides causing renal tubular obstruction, may contribute to rhabdomyolysis-induced renal injury through a heme-iron-mediated lipid peroxidation process. In the present study, we compared the combined therapy of a lipid peroxidation inhibitor, 21-aminosteroid (21-AS) and fluid-alkaline-mannitol (FAM) diuresis with either of them alone to determine the efficacy of the combination therapy and to delineate the roles of lipid peroxidation and cast formation.

Methods and results. Employing Raman spectroscopy, we confirmed in vitro the ability of 21-AS to inhibit iron-induced fatty acid peroxidation. 21-AS was then administered to rats developing renal failure from glycerol-induced rhabdomyolysis. Although 21-AS inhibited rhabdomyolysis-induced plasma and renal lipid peroxidation, renal protection was incomplete. Administration of FAM to inhibit cast formation afforded a better renal protection. However, when these therapies were combined to inhibit both lipid peroxidation and cast formation, there was a synergistic renal functional protection. This was accompanied by a maximum inhibition of renal and plasma lipid peroxidation, as well as, renal tubular necrosis and cast formation. Compared to combination therapy, FAM therapy alone, despite identical volume, was accompanied by a higher tubular necrosis and cast formation.

Conclusions. That combining a lipid peroxidation inhibitor with fluid-alkaline diuresis in rhabdomyolysis further lowers renal lipoperoxidation, tubular necrosis and cast formation and synergistically limits renal dysfunction (i) supports a role for lipid peroxidation in the pathophysiology of rhabdomyolysis ARF, (ii) underscores the role of intratubular heme retention, a cause for tubular obstruction as well a source for prodigious amount of iron, likely involved in the lipid peroxidation, and (iii) raises the possibility of interactions between non-oxidant and oxidant mechanisms.

Key words: acute renal failure; 21-aminosteroid; heme-proteins; lipid peroxidation; rhabdomyolysis; Raman spectroscopy

Introduction

Following rhabdomyolysis, inordinate amounts of myohaemoglobin are released into the systemic circulation and its prompt discharge into the renal tubules sets the stage for the initiation of renal injury process [1,2]. Both oxidant and non-oxidant mechanisms are considered to be important in the complex pathophysiology of myohaemoglobin-mediated renal injury of rhabdomyolysis [1-7]. Hypovolaemia and metabolic acidosis facilitate tubular precipitation of myohaemoglobin and therefore, form the basis for fluid-alkaline-diuresis therapy [1,2,6]. Myohaemoglobin, besides causing tubular obstruction, may contribute to tubular epithelial cell injury through a probable heme-iron-mediated lipid peroxidation mechanism. It is conceivable that the latter process may further compromise the dynamics of tubular fluid flow, interfering with the complete clearance of tubular heme proteins, and thus initiating a vicious cycle. Even in the absence of heme-protein precipitation, which can be achieved by vigorous fluid-alkaline diuresis, tubular injury may still occur because of the continued bathing of the tubular cells in myohaemoglobin filtrate. The latter may facilitate tubular cell heme-loading which along with the attendant release of catalytically active iron [7] may potentially instigate cellular lipid peroxidation and injury. That both oxidant and non-oxidant mechanisms are important in rhabdomyolysis-induced renal injury is suggested by the finding that neither diuresis nor antioxidants therapy alone is completely protective [3,5,7].

In this study, we attempted to define the relative significance of the two above mentioned main mechan-
isms, specifically, one related to cast formation and the other related to oxidative injury. With regard to the latter, we were particularly interested to examine the role of lipid peroxidation that often accompanies rhabdomyolysis-induced acute renal injury [3,5]. Recent cell culture studies suggest a role for lipid peroxidation in hydrogen peroxide-induced renal epithelial cell injury [8]. Although both excessive hydrogen peroxide generation and lipid peroxidation have been demonstrated in kidneys sustaining injury from rhabdomyolysis [4,5], the role of accompanying lipid peroxidation in rhabdomyolysis-induced renal injury is not well defined.

21-aminosteroid (U-74006 F) the agent employed to inhibit lipid peroxidation in this study is a relatively new group of antioxidant that potently inhibits lipid peroxidation, primarily by scavenging lipid peroxyl and phenoxyl radicals [9] and has been demonstrated to potently inhibit oxidant-induced renal tubular cell lipid peroxidation and injury [8]. Furthermore, 21-aminosteroid and its potent analog 2-methyl aminochroman have been recently reported to modestly reduce renal injury in the rhabdomyolysis model [10,11]. Although these recent studies imply a therapeutic potential for these compounds, the important question whether such therapy is better than existing fluid-alkali-mannitol therapy has not been addressed previously. Moreover, since myohaemoglobin-induced renal injury in rhabdomyolysis is probably a combination of oxidant and non-oxidant mechanisms, another important question whether combining 21-aminosteroid with fluid-alkali-mannitol therapy provides additional renal protection has also not been addressed.

In the present study, employing Raman spectroscopy for the first time, we confirmed the capacity of 21-aminosteroid to directly inhibit iron-induced lipid peroxidation. We then examined in the glycerol-induced rhabdomyolysis model, the effect of (i) inhibiting renal lipid peroxidation by 21-aminosteroid, (ii) inhibiting the tubular cast formation by vigorous fluid-alkali-mannitol diuresis, or (iii) inhibiting both lipid peroxidation and cast formation by combining these two therapies on myohaemoglobin-induced renal injury. The data that both lipid peroxidation and cast formation contribute to the rhabdomyolysis-associated renal injury and the combination therapy affords more than an additive renal protection suggest that such combination therapy, unlike either of them alone, may offer the best renal outcome. Such synergistic renal protection with the combination therapy may also suggest the possible existence of interactions between oxidant and non-oxidant mechanisms, potentially compounding the severity of rhabdomyolysis-induced renal injury.

Methods

Effect of 21-aminosteroid on iron-induced fatty acid peroxidation: Raman spectroscopy

Raman measurements were carried out by a system consisting of a triple-spectrometer, a micromate microscope, a tunable excitation filter and a charge coupled device detector, all obtained from Spex Inc. (Edison, NJ). A Spectra-Physics (Mountain View, CA) Kr + laser (647.1 nm) was used as an excitation source. For controlling the CCD detector and analysing the Raman data, a Spex model DM 3000 data system with an IBM PC was used.

The following samples were prepared for the Raman spectroscopic investigation to study the effect of 21-aminosteroid on iron-dependent lipid peroxidation. Sample A consisted of 5 μl of 99% linolenic acid, 35 μl of 100% ethanol, and 10 μl of deionized water (DIW). Spectroscopy was carried out immediately on this vortexed sample placed on a quartz plate, to obtain the spectrum of pure linolenic acid, i.e., without the possible autooxidation from incubation. The remaining samples (below) were subjected to Raman spectroscopy following a 16 h incubation at 37°C. Sample B (time-control) consisted of 5 μl of 99% linolenic acid, 35 μl of 100% ethanol, and 5 μl of DIW. Sample C (with iron) contained 5 μl of linolenic acid, 5 μl of 2 mM FeCl₂ solution in DIW, 35 μl of ethanol, and 5 μl of DIW. Sample D (with iron plus 21-aminosteroid) consisted of 5 μl of linolenic acid, 5 μl of 2 mM FeCl₂ in DIW, 5 μl of 1 mM 21-aminosteroid in ethanol, and 30 μl of ethanol. Sample E (with iron plus BHT) consisted of 5 μl of linolenic acid, 5 μl of 2 mM FeCl₂ in DIW, 5 μl of 2% butylated hydroxytoluene in ethanol, and 30 μl of ethanol. BHT was included as a known inhibitor of lipid peroxidation.

Effect of 21-aminosteroid on glycerol-induced acute renal failure and lipid peroxidation parameters

Two of three groups of overnight dehydrated (18 h) male Sprague–Dawley rats of similar weight (250–275 g), serum creatinine, haematocrit, and serum protein were injected i.m. with 10 ml/kg of 50% glycerol, half each into each hind muscles under mild anaesthesia (pentobarbital sodium, 20 mg/kg, i.p.). One of these glycerol injected groups (GARF +21-AS), received 21-aminosteroid; first of three doses (10 mg/kg) was administered by tail vein 15 min after the glycerol, and second and third at 4 h intervals. The other group (GRAF) injected with glycerol received vehicle of 21-aminosteroid, 0.15 ml/100 g body weight of 0.01 M HCI, 3 times in an identical manner. The third group, the sham control, received i.m. normal saline instead of glycerol and the above vehicle. To minimize variability in water drinking, rats were gavaged with 0.5 ml/100 g of water twice at 2 and 4 h after glycerol. Free water intake was allowed 6 h after glycerol. Rats were reanaesthetized at 24 h after glycerol, blood samples were drawn from vena caval puncture, plasma separated immediately stored at −84°C. Renal cortices were rapidly dissected out on a petri dish with ice-cold normal saline, rinsed, blotted, weighed and frozen at −84°C.

Haematocrit (microcapillary method), plasma protein (refractory method), and serum creatinine (Beckman autoanalyser, Somerser, NJ) were determined.

Evidence for lipid peroxidation in the kidney samples was sought by measuring two products of lipid peroxidation—malondialdehyde and conjugated diene—by the assay methods described in our previous studies [12,13]. Briefly, renal cortex was homogenized in 100 mM potassium chloride containing 3 mM EDTA and malondialdehyde in the supernatant was measured in the presence of antioxidants—EDTA (3 mM) and butylated hydroxytoluene (2% BHT, 10 μl/ml)—to minimize procedural lipid peroxidation. Malondialdehyde was measured as thiobarbituric acid react-
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Effect of combining 21-aminoosteroid with fluid-alkaline-mannitol therapy on glycerol-induced acute renal failure and lipid peroxidation parameters

Effect of combining 21-aminoosteroid on iron-induced fatty acid peroxidation: Raman spectroscopy

Materials

Results
induced linolenic acid spectral changes similar to 21-aminosteroid. FeCl₂-induced linolenic acid peroxidation in this experimental setting was further verified by the thiobarbituric acid reaction (data not shown). These results directly demonstrate the ability of 21-aminosteroid to inhibit iron-dependent peroxidation of unsaturated fatty acid.

**Effect of 21-aminosteroid on glycerol-induced acute renal failure and lipid peroxidation parameters**

Despite similar degree of dehydration and renal function, i.m. administration of glycerol but not saline was accompanied by fairly severe acute renal failure. At 24 h, serum creatinine was 2.5 ± 0.3 in the GARF group, compared to 0.50 ± 0.1 mg/dl in the sham control. Although 21-aminosteroid administration inhibited rhabdomyolysis-induced lipid peroxidation, based on the plasma and kidney homogenate levels of malondialdehyde and conjugated dienes, renal functional protection, although significant, was far from complete: the serum creatinine in the GARF + 21-AS was 1.67 ± 0.15 compared to 0.5 ± 0.1 mg/dl in the sham control (Table 1).

The above finding of modest renal protection by 21-aminosteroid was confirmed in a separate study in which GFR was measured by the 125I iothalamate clearance method (Figure 2). GFR at 24 h was 1.70 ± 0.12 ml/min in the sham rats injected with saline that was strikingly low in the glycerol injected group, at 0.059 ± 0.33 ml/min. Confirming the serum creatinine data, 21-aminosteroid therapy, although significantly improving GFR (0.264 ± 0.082 ml/min), it was

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<th>Table 1. Measurements in the sham rats, glycerol-injected rats (GARF) and glycerol-injected rats receiving 21-aminosteroid (GARF + 21-AS)</th>
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*P<0.05 versus the rest, **versus control; ***post-dehydration; n=4 in each group.
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ineffective in maintaining the GFR in any level closer to the normal level (Figure 2). Renal blood flow at 24 h was significantly reduced following glycerol-induced rhabdomyolysis, but was significantly better with the administration of 21-aminosteroid (Figure 2).

Plasma CPK level, an index of muscle injury, was elevated following glycerol-injection compared to saline (33.3 ± 12.5 versus 12.5 ± 2.5 Sigma U/ml). 21-Aminosteroid therapy had no significant effect on the glycerol-induced CPK elevation (29.5 ± 1.5 Sigma Units/ml), thus discounting any important effect of antioxidant therapy on muscle injury in this protocol.

Effect of combining 21-aminosteroid with fluid-alkaline-mannitol therapy on glycerol-induced acute renal failure and lipid peroxidation parameters

Renal function with 21-aminosteroid therapy alone was again significantly better than the untreated glycerol injected control (Figure 3). The group treated with fluid-alkali-mannitol alone had a slightly better renal function than the group treated with 21-aminosteroid alone: the serum creatinine was 1.80 ± 0.2 mg/dl compared to 2.1 ± 0.25 mg/dl in the 21-aminosteroid alone group (Figure 3). The respective BUN values were 83 ± 10 and 94 ± 13 mg/dl (Figure 3).

The striking finding of this study was the remarkable renal protection when therapies were combined. Compared to the 2.58 ± 0.20 mg/dl of serum creatinine in the rhabdomyolysis control (GARF), serum creatinine rose only to 0.82 ± 0.10 with combination therapy (Figure 3). The difference between the mean serum creatinine in the GARF and GARF + 21-AS + FAM was 1.78, which exceeded the combined difference of 1.24 from the individual therapies. Because of the curve-linear relation between serum creatinine and GFR, the lower serum creatinine in the combined therapy group may even represent a greater preservation of renal function than appreciated by serum creatinine measurements. Findings similar to serum creatinine was also demonstrable with BUN values (Figure 3). Thus, the renal protection from the combination therapy was more than additive.

Such synergistic renal functional protection with combined therapy was accompanied by an almost complete inhibition of tubular cast formation as well as maximum reduction in tubular necrosis (Figure 4B). This group also had the maximum inhibition of systemic and renal lipid peroxidation (Figure 5). Plasma malondialdehyde was 3.65 ± 0.72 nmol/ml, the lowest of all the groups. The rest were 6.13 ± 0.54 in the GARF, 5.25 ± 0.22 in the GARF + 21-AS (P < 0.05 versus GARF) and 3.92 ± 0.34 in the GARF + FAM (P < 0.05 versus GARF and GARF + 21-AS).

The renal protection of 21-aminosteroid therapy appeared to have derived from its ability to limit lipid peroxidation (Figure 5) and cell necrosis (Figure 4B) and not from reducing cast formation (Figure 4B), since the latter score was slightly higher than the glycerol-treated control. The fluid-alkali-mannitol group received the same solution in an identical manner; yet, in the absence of 21-aminosteroid administration, there was over a three-fold increase in myohaemoglobin cast retention and over a two-fold increase in tubular necrosis (Figure 4B). Remarkable also was that fluid-alkali-mannitol therapy, which predictably reduced the tubular heme-protein load based on tubular cast score (Figure 4B), was also attended by a reduction in renal lipid peroxidation (Figure 5).

Discussion

In this study, inhibition of lipid peroxidation by administering 21-aminosteroid during the evolution of heme-protein-induced renal injury was accompanied by a modest renal improvement. 21-Aminosteroid administration limited tubular necrosis but not the formation of casts. Although still incomplete, renal function was slightly better preserved with fluid-alkali-mannitol diuresis that appeared to have been due its effect on reducing the cast formation, and not tubular necrosis. In contrast, combination therapy aimed at inhibiting both lipid peroxidation and preventing cast formation was accompanied by a synergistic renal functional protection that was also attended by a maximum

![Fig. 3. Serum creatinine and BUN were determined before and 24 h after glycerol in GARF, GARF + 21-AS, GARF + FAM, and GARF + FAM + 21-AS. **: Factors for conversion to SI unit is 88.4 for creatinine (μmol/l) and 0.357 for BUN (mmol/l). *: P < 0.05 versus GARF. †: P < 0.05 versus the rest. The GARF group received the vehicle, GARF + 21-AS group 21-aminosteroid, the GARF + FAM group sodium bicarbonate in saline and mannitol and the GARF + FAM + 21-AS group above therapies combined.

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inhibition of lipid peroxidation as well as tubular necrosis and cast formation.

While there is little doubt that heme-proteins play a central role in the pathogenesis of rhabdomyolysis-induced acute renal failure, accruing evidence suggests that besides causing obstructive cast and direct tubular toxicity (non-oxidant mechanism), heme-protein, by being the source for an enormous amount of catalytic iron, can contribute to renal injury through a lipid peroxidation mechanism (oxidant mechanism) [3–5,7]. The findings in this study, besides confirming the roles of both oxidant and non-oxidant mechanisms in the pathogenesis of rhabdomyolysis, suggest a relative preponderance for the role of non-oxidant mechanism. However, therapy against the non-oxidant mechanism alone was inadequate in preserving the renal function. Such inadequacy is probably reflective of the inability of fluid-alkali-mannitol to prevent heme-protein-induced tubular necrosis. Heme-protein filtrate, although diluted from diuresis, is still capable of generating excessive amount of catalytically active iron as recently demonstrated [7]. Such excess iron, although far less than in the untreated glycerol group, could still instigate lipid peroxidation and cell necrosis. This possibility is supported by the finding here that, compared to fluid-alkali-mannitol therapy alone, addition of 21-aminosteroid to fluid-alkali-mannitol further reduced the extent of tubular lipid peroxidation, necrosis and renal dysfunction function. It should be noted however, that, although addition of 21-aminosteroid further enhanced the renal protectiveness of alkaline-mannitol-diuresis, its therapy alone did not offer any advantage over the conventional fluid-alkali-mannitol diuresis. The upper end of the recommended dose of 21-aminosteroid employed here was adequate enough to completely suppress both the plasma and the renal lipid peroxidation (Table 1) and therefore, further increase in the dose of 21-aminosteroid is not expected to bring any additional renal protection.

Fluid-alkali-mannitol alone was effective in reducing lipid peroxidation and this may relate to the dilution of tubular heme-protein concentration rather than to reduced tubular damage, since tubular necrosis was still a prominent feature in the diuresis group (Figure 4B). Alternatively, presence of cast in tubular

Fig. 4. (A) Two indices were scored blindly under light microscopy on the formalin-fixed hematoxylin and eosin stained kidney sections, one, for tubular cast indicated above by the thin arrow and the other, for frank tubular necrosis, indicated by the thick arrow. (B) Renal histological score of tubular necrosis and tubular cast formation in the four groups of glycerol-injected rats receiving vehicles, 21-aminosteroid, fluid-alkali-mannitol or both 21-aminosteroid and fluid-alkali-mannitol. *: P<0.05 versus GARF. †: P<0.05 versus the rest.

Fig. 5. Lipid peroxidation products in the renal cortical homogenate of the four groups of glycerol-injected rats receiving vehicles, 21-aminosteroid, fluid-alkali-mannitol or both 21-aminosteroid and fluid-alkali-mannitol. *: P<0.05 versus GARF. †: P<0.05 versus GARF + FAM.
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Lumens may be a prerequisite to instigate the oxidative cellular injury. The previous demonstration that direct addition of iron or heme-protein induces cellular lipid peroxidation and injury would however, argue against this [7,10].

The rhabdomyolysis model in the rat, in many ways similar to the human syndrome, is studied here (as by many others) with prior dehydration [3–5]. Prior dehydration allows the full expression of the renal injury, particularly the cast formation, compared to the non-dehydrated model [7]. Furthermore, dehydration is not an uncommon occurrence in the clinical setting of rhabdomyolysis [1,2]. Therapies in this study were instituted 15 min after the initiation of muscle injury; a clinically relevant question is whether further delay in the institution of combination therapy, especially when conventional therapy is ineffective, still affords renal protection following rhabdomyolysis.

Although lipid peroxidation is well recognized to occur during the renal injury, particularly associated with oxidative stress, its direct role in the causation of renal injury is less well defined. Recent studies suggest a role for lipid peroxidation in certain in vitro and in vivo settings of renal injury [7,12,13,15], but data from others indicate that its occurrence does not necessarily mean that it is causal to the co-existing pathophysiology [16,17]. The present data that the inhibition of lipid peroxidation by an anti-oxidant or by an alkaline-diuresis, the latter by reducing the concentration of catalytically active iron, results in renal protection further defines a role for lipid peroxidation in rhabdomyolysis-induced renal injury.

A number of studies support the view that it is the catalytically active iron released from heme-protein that mediates the renal injury in this model [3,5,7]. An important mechanism of iron-induced tissue injury is its well-known ability to induce lipid peroxidation [18,19]. Indeed, iron-induced lipid peroxidation in vitro is a model that has been extensively employed in the study of lipid peroxidation [18,19]. It was in one such model that the potency of 21-aminosteroid to inhibit iron-induced lipid peroxidation was first demonstrated [20]. Subsequent studies in our laboratory and that of others have shown that the ability of 21-aminosteroid to inhibit lipid peroxidation was not limited for iron [7,9]. The ability of 21-aminosteroid to inhibit lipid peroxidation resides in the amino group, which by combining with lipid radicals limit the propagation of lipid peroxidation [9,20].

The present data from the Raman spectroscopic study confirms the ability of 21-aminosteroid to directly inhibit iron-induced lipid peroxidation. Although, Raman spectrum has been recorded previously for fatty acids of varying unsaturation [14], to our knowledge this is the first time this methodology is applied to study the spectral changes associated with the peroxidation of a lipid [21]. The application of Raman spectroscopy in the biomedical field is in its infancy and currently limited to the in vivo study of atherosclerosis and the diagnosis of breast cancer [21].

We have chosen polyunsaturated linolenic acid rather than arachidonic acid to complete the present study because we found that the latter was less susceptible to iron-induced spectral changes, i.e. peroxidation, the reason for which is not clear at present. 21-Aminosteroid, like BHT, was able to directly attenuate the iron-induced linolenic acid peroxidation. The present finding that 21-aminosteroid inhibits fatty acid peroxidation is consistent with our recent report that 21-aminosteroid inhibited H2O2-induced renal epithelial cell F2-isoprostane production. F2-isoprostanes are newly identified arachidonic peroxidation products. When infused into the kidneys, they induce intense renal vasoconstriction [22]. In the present study, 21-aminosteroid administration was attended by preservation of renal blood flow, compared to untreated rats with rhabdomyolysis. Increased F2-isoprostane production may be one factor for myohaemoglobin-induced renal vasoconstriction.

The fluid-alkali-mannitol protocol, adopted here based on clinical practice, ensured that there was active diuresis with effective alkalinization of the urine (pH ranged 7–7.5). Although mannitol is considered to have hydroxyl radical scavenging property, recent studies specifically addressing this issue in the setting of rhabdomyolysis-renal injury demonstrate that the efficacy of mannitol in rhabdomyolysis is most probably due to its diuretic effect rather than an antioxidant effect [7]. The clinical rationale for the systemic alkalinization in rhabdomyolysis has been experimentally substantiated [6]. The benefit includes not only reducing the heme-protein precipitation but also the formation of toxic met-hemoglobin. However, no study has previously combined alkalinization and diuresis with

![Diagram](https://example.com/diagram.png)

Fig. 6. This simplified scheme, based on existing data and the findings of the present study, underscores the role of both oxidant and non-oxidant mechanisms in the mediation of myohaemoglobin-induced tubular injury. Data in this study also raises the possibility of confounding interactions of these two mechanisms; a possibility that strengthens a case for combining therapy in rhabdomyolysis-related renal injury.
Antioxidant therapy in a rhabdomyolysis model. In a previous experimental study on rhabdomyolysis-induced acute renal failure, combining desferrioxamine with mannitol-diuresis but without alkalinization was accompanied by an additive renal protection with minimal tubular cell necrosis. In that study, although desferrioxamine therapy alone virtually eliminated the enormous increase in catalytically active urinary iron, renal protection was incomplete. This finding relates to our present one, i.e., 21-aminosteroid alone effectively inhibited lipid peroxidation, but it did not provide complete renal protection. Data on tubular casts in the present study suggest that the reason for incomplete renal protection with antioxidants alone is likely due to inability to eliminate the tubular cast formation. Thus, even if iron-mediated lipid peroxidation is inhibited either by 21-aminosteroid or by desferrioxamine, tubular casts can continue to compromise renal function.

Although kidneys end up serving a "sacrificial role," while trying to rid the systemic circulation off the flagitious myohemoglobin, the systemic nature of rhabdomyolysis is demonstrated by the finding that lipid peroxidation products were elevated in the systemic circulation and not merely confined to the kidney. This relates to the previous observation that catalytically active iron was markedly elevated not only in the urine but also in the plasma [7]. Such systemic nature of iron release and lipid peroxidation provide further indirect evidence for a link between lipid peroxidation and heme-protein released catalytic iron.

Both cast formation and oxidant-induced lipid peroxidation appear capable of contributing to renal injury, independently and interdependently. That the combination therapy employed here affords more than an additive renal protection along with maximum suppression of cast formation and lipid peroxidation could best be explained by the existence of confounding interactions between these two mechanisms (Fig. 6). This notion, along with the demonstrable synergistic efficacy of combined therapy to limit renal dysfunction in this study, suggests that concurrent therapy aimed at both the oxidant and non-oxidant mechanism may yield the maximum protection against rhabdomyolysis-induced acute renal failure.

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