SHOCK, Vol. 24, No. 2, pp. 139–144, 2005

HIGH-DOSE VITAMIN C INFUSION REDUCES FLUID REQUIREMENTS IN THE RESUSCITATION OF BURN-INJURED SHEEP

Michael A. Dubick,* Chad Williams,† Geir I. Elgjo,† and George C. Kramer†

**U.S. Army Institute of Surgical Research, San Antonio, Texas; and †University of Texas Medical Branch, Galveston, Texas*

Received 16 Mar 2005; first review completed 4 Apr 2005; accepted in final form 2 May 2005

ABSTRACT Fluid resuscitation to maintain adequate tissue perfusion while reducing edema in the severely burned patient remains a challenge. Recent studies suggest that reactive oxygen species generated by thermal injury are involved in edema formation associated with burn. The present study tested the hypothesis that adding a free radical scavenger to the resuscitation fluid would reduce total fluid requirements in the treatment of severe thermal injury. Anesthetized chronically instrumented sheep received a 40% total body surface area full-thickness flame burn. At 1 h after injury, animals were resuscitated with lactated Ringer's (LR, n 14) as control, LR containing high doses of vitamin C (VC, n 6), 1000 mOsM hypertonic saline (HS, n 7), or 1000 HS containing VC (HS/VC, n 7) in coded bags so that investigators were blinded to the treatment. Fluids were infused at an initial Parkland rate of 10 mL/kg/h, adjusted hourly to restore and maintain urine output at 1 to 2 mL/kg/h. Sheep in the VC or HS/VC group received 250 mg/kg VC in the first 500 mL of LR or HS, and then 15 mg/kg/h thereafter. Hemodynamic variables and indices of antioxidant status were measured. At 48 h postburn, sheep were euthanized, and heart, liver, lung, skeletal muscle, and ileum were evaluated for antioxidant status. All fluid resuscitation regimens were equally effective in restoring cardiac output to near baseline levels; no treatment effects were apparent on arterial pressure or heart rate. VC infusion significantly reduced fluid requirements and, therefore, net fluid balance (fluid in, urine out) by about 30% at 6 h and about 50% at 48 h in comparison with the LR group ($P < 0.05$). HS and HS/VC reduced fluid requirements by 30% and 65%, respectively, at 6 h, but the volume-sparing effect of HS was not observed after 36 h and that of HS/VC was lost after 12 h. Plasma total antioxidant potential increased about 25-fold ($P < 0.05$) at 2 and 3 h in response to VC infusion compared with the LR and HS groups, and remained about 5- to 10-fold higher throughout the rest of the study. VC infusion also prevented the 4-fold increase in plasma thiobarbituric acid reactive substances seen in the LR group early after burn ($P < 0.05$). Tissue antioxidant status was similar between groups. In this sheep burn model, continuous high-dose VC infusion reduced net fluid balance, reduced indices of plasma lipid peroxidation, and maintained overall antioxidant status in comparison with standard-of-care LR treatment.

KEYWORDS Vitamin C, ascorbic acid, fluid resuscitation, burns, sheep, antioxidants

INTRODUCTION

Thermal injury affects about 2 million people a year in the United States, with 50,000 to 100,000 people requiring hospitalization (1, 2). Previous assessment of combat casualties in past military conflicts reported that thermal injuries constituted 5% to 15% of casualties in conventional warfare (3), and a recent Army Conference on military medicine indicated that the incidence of burns could be expected to increase in future conflicts (4). Thus, optimal resuscitation of thermally injured combat casualties in forward areas of the battlefield is not a trivial matter and must be defined. Thermal injuries larger than 15% of the total body surface area (TBSA) require fluid resuscitation to prevent the development of hypovolemic shock or renal failure (1, 5). It is also recognized that hypovolemia can develop rapidly after burn and progress over the next 24 h despite fluid resuscitation (5).

The importance of early, adequate fluid resuscitation in burn patients is well recognized, but treatment must be carefully titrated to avoid exacerbating the interstitial edema associated with the burn that could lead to poor tissue perfusion and organ failure (1). Although this desired balance in fluid resuscitation is practically achievable in most burn centers, far-forward treatment of the thermally injured combat casualty offers additional challenges, such as the environmental and tactical conditions of the battlefield, the presence of mass casualties, and/or logistic constraints that preclude the availability of large volumes of crystalloid fluids for resuscitation. Typically, fluid resuscitation of patients with thermal injuries is based on providing 2 to 4 mL/kg per percentage of burn of lactated Ringer's (LR) to maintain urine output over 1.0 mL/kg/h, with one-half of the fluid expected to be infused over the first 8 h and the remaining one-half infused over the next 16 h (5 7). However, such treatment is only partially effective because much of the infused fluid leaks into the extravascular space, resulting in edema and its related morbidity (8, 9). Thus, the development of small volume resuscitation strategies that could reduce the fluid requirements for the treatment of thermal injuries without impacting outcome would be beneficial in civilian burns, and would offer a tremendous logistic advantage to the military.

Previous studies have indicated that this developing hypovolemia and immediate need for fluid resuscitation appear to be related to the increased vascular permeability and capillary leakage observed physiologically and morphologically after thermal injury (1). Recent studies also suggest that reactive oxygen species (ROS) generated by thermal injury are involved in the increased microvascular permeability, edema formation, and tissue damage associated with burn (10 14). ROS have been implicated in much of the complex pathophysiologic processes

Address reprint requests to Dr. Michael Dubick, U.S. Army Institute of Surgical Research, 3400 Rawley E. Chambers Avenue, San Antonio, TX 78234-6315. E-mail: Michael.Dubick@amedd.army.mil.

This work was supported in part by a Shriners Hospital grant (no. 8720).
DOI: 10.1097/01.shk.0000170355.26060.e6

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 AUG 2005	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
High dose vitamin C infusion reduces fluid requirements in the resuscitation of burn injured sheep.	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Dubick M. A., Williams C., Elgjo G. I., Kramer G. C.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA FOrt Sam Houston, TX 78234	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a REPORT	b ABSTRACT	c THIS PAGE			
unclassified	**unclassified**	**unclassified**	**SAR**	**6**	

after burn that are characterized by an inflammatory response that activates neutrophils, stimulates complement, induces intravascular hemolysis, and releases vasoactive substances, proinflammatory cytokines, catabolic hormones, and other inflammatory mediators such as histamine, bradykinin, and prostaglandins (15). Additional support for the role of ROS in burns was observed by a number of investigators who found that depletion of neutrophils in rats, or treating them with a combination of catalase and superoxide dismutase, reduced the acute lung injury seen in untreated animals after a cutaneous burn (15, 16). It has also been observed that endogenous antioxidants are affected by thermal injury. For example, significant elevations in glutathione and ascorbic acid (vitamin C) were observed in the subcutaneous interstitial fluid immediately after thermal injury to rats (17), and in other studies, plasma vitamins C, E, and β-carotene concentrations were low upon admission of burn patients to the hospital (18). Therefore, it was suggested that antioxidants may be useful in the treatment of thermal injuries.

Over the past decade, a series of studies have focused on the potential benefit of large doses of vitamin C in reducing fluid requirements and tissue edema associated with a cutaneous thermal burn (19 26). The present study tested the hypothesis that adding a free radical scavenger, such as vitamin C, to the resuscitation fluid would reduce total fluid requirements in the treatment of severe thermal injury. A second study was conducted to determine whether high-dose vitamin C would augment the fluid-sparing activity of hypertonic saline (HS) observed previously in the treatment of hemorrhagic and burn shock (27 30).

MATERIALS AND METHODS

Animal preparation

Adult, female Merino sheep (28 42 kg) were anesthetized and mechanically ventilated under 1.5% halothane anesthesia. They were then instrumented with indwelling vascular catheters in the abdominal aorta for recording blood pressure and heart rate and for drawing arterial blood samples and in the inferior vena cava for infusion of maintenance fluid during surgery and for infusion of resuscitation fluids after burn. A 7-French Swan Ganz catheter (Baxter Healthcare, Irvine, CA) was positioned in the pulmonary artery for measurement of cardiac output by thermodilution and for drawing blood samples for mixed venous blood gas analysis. Experiments were performed 5 to 7 days after surgery.

Protocol

On the day before burn injury, the vascular catheters were attached to pressure transducers (Baxter Pressure Monitoring kit; Baxter Healthcare) connected to a patient monitor (model 78901A; Hewlett-Packard, Andover, MA) for continuous monitoring of hemodynamic variables, and to condition the sheep to the experimental conditions. On the day of burn injury, sheep were anesthetized with halothane and received 0.3 mg buprenorphine as added analgesia, they were weighed, and a Foley catheter was placed in their bladder and secured for measurement of urinary output. Sheep then received a 40% TBSA full-thickness flame burn as previously described (29, 30). Adequate burn depth was confirmed by blanching and contraction of the burned skin. Sheep were then allowed to recover from the anesthesia and were given buprenorphine before the burn and every 12 h thereafter. Animals were denied water for the duration of the 48-h experiment as intravenous fluid was provided to maintain urinary output within normal range, but they were allowed food after 24 h. The experiments were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals and the study was approved by our Institutional Animal Care and Use Committee.

Treatment groups

We performed two concurrent studies with identical animal preparations and protocol except for treatment. Study one was performed only with isotonic fluids. At 1 h after injury, animals were resuscitated with LR (273 mOsm/L, n = 9) as the standard of care, or LR containing a high dose of vitamin C (10 g/500 mL, ~386 mOsm/L, in the first 500 mL and followed by regular LR). This is a dose of about 250 mg/kg in the first 500 mL and was followed by continued infusion of 15 mg/kg/h thereafter in a second small volume constant infusion. All solutions were in coded bags so that investigators were blinded to the treatment. Fluid in both groups was infused initially, according to the standard Parkland formula, at a rate of 10 mL/kg/h for the 40% TBSA burn injury, but then the LR infusion rate was adjusted hourly to restore and maintain urine output at 1 to 2 mL/kg/h. A set algorithm defined how the infusion rate was altered based on urine output. The algorithm was designed to maintain urinary output in the normal range for sheep (1 2 mL/kg per h), thus infusion rate was increased when the last hour's urinary output was below this range and was decreased when it was above it. The specifics for infusion are contained in a decision table that has been published (29).

In the second study, three groups of sheep were resuscitated with isotonic LR (n = 5), 1000 mOsM HS (n = 5), or 1000 mOsM HS containing vitamin C as indicated above (n = 6). It is noted that this LR group treatment is identical to that of the first study. Fluid infusion, adjusted to maintain urine output at 1 to 2 mL/kg/h, was conducted as described above. In this way, a consistent, individualized treatment regimen was achieved in both studies.

Net fluid accumulation was defined as cumulative infused fluid volume minus cumulative urine output, and was measured at each hourly interval starting at 1 h postinjury when resuscitation was initiated. Fluid sparing was defined as the difference in net fluid accumulation between the LR group and the other treatment groups at any time point after burn.

Hemodynamic variables, including mean arterial pressure, heart rate, central venous pressure, pulmonary arterial pressure, pulmonary arterial wedge pressure, and body temperature, were recorded in triplicate at preburn baseline, and after burn injury at 0.5-h intervals until 6 h, at 1-h intervals from 6 to 24 h, and at 3-h intervals from 24 to 48 h. Cardiac output was measured in triplicate by injection of 10 mL of ice-cold 5% dextrose at the same time points, and was calculated by a cardiac output computer (model 9520A; American Edwards Laboratories, Edwards Critical Care Division, Irvine, CA).

Arterial blood samples were collected for determination of hematocrit and osmolality by standard clinical procedures. Plasma ascorbic acid concentrations were determined indirectly by assessing changes in the total antioxidant potential as described by Benzie and Strain (31). Plasma thiobarbituric acid reactive substances (TBARS) concentrations were determined as an index of lipid peroxidation according to the spectrophotometric method described by Naito et al. (32). At 48 h postburn, sheep were euthanized, and heart, liver, lung, skeletal muscle, and ileum were collected and assayed for antioxidant potential and TBARS as described above, and for the activities of the antioxidant enzymes glutathione peroxidase, glutathione reductase, and copper-zinc and manganese superoxide dismutase (33). Tissue oxidized and reduced glutathione concentrations were determined enzymatically as described by Anderson (34).

Data in graphs are expressed as mean ± SEM. The ''standard of care'' LR treatment was identical in the two studies and these data were pooled for comparison with the other novel treatment groups. Data were analyzed for the four different treatments using a repeated measure analysis of variance. *Post hoc* tests between treatment groups used Tukey's HSD test corrected for multiple comparisons. Effects and interactions were assessed at the 0.05 level of significance.

RESULTS

All animals survived the 48-h experimental period. Burn injury reduced cardiac output, expressed as the percentage of change in cardiac index from baseline, about 40% to 50% in all groups (Fig. 1). All fluid resuscitation regimens were equally effective in restoring cardiac output to near baseline levels (Fig. 1). Hematocrit increased after burn injury due to loss of plasma volume and was returned to preburn levels in all groups, showing adequate restoration of plasma volume with all treatments (Fig. 2).

Blood pressures and heart rates exhibited changes after burn injury and during resuscitation as we have previously reported in detail (29, 30) In this study, there were no apparent or statistically significant effects of treatment on hemodynamics in these groups, all of which received volume titrated to maintain normal urinary outputs. Mean arterial pressure exhibited a small postburn increase of 5 to 10 mmHg, which did not reach statistical significance in any group. Heart rate rose significantly after burn 10 to 30 bpm and then fell to near baseline level by 12 h after

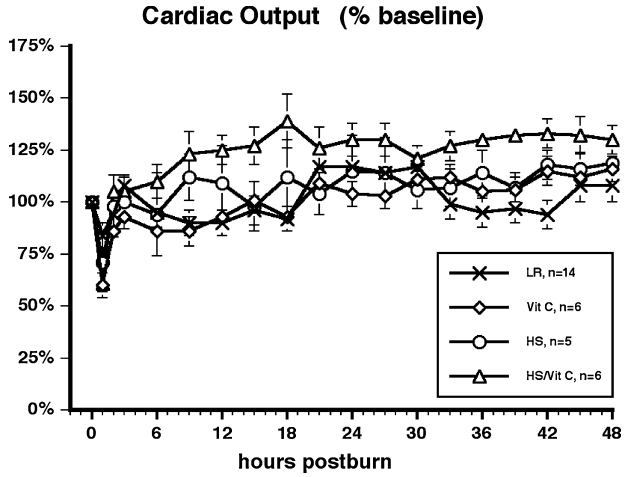

FIG. 1. **Cardiac output after thermal injury in sheep resuscitated with LR, high-dose vitamin C, HS or HS with high-dose vitamin C**. See text for description of vitamin C dosing. Data expressed as mean ± SEM.

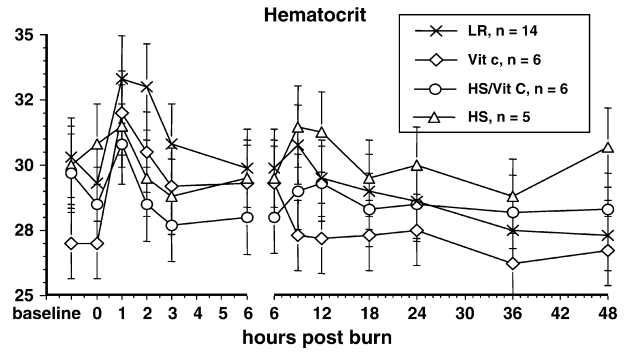

FIG. 2. **Hematocrit after thermal injury and fluid resuscitation in sheep**. Data are shown for the first 6 h after burn and from 6 to 48 h after burn. Data are expressed as mean ± SEM.

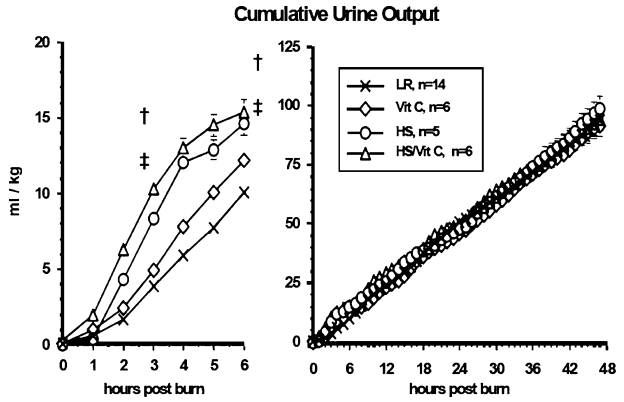

FIG. 3. **Cumulative urine output during the first 6 h after thermal injury and over the entire 48-h experimental period**. Data expressed as mean ± SEM. *Vitamin C versus LR, †HS/vitamin C versus LR, ‡HS versus LR at $P < 0.05$.

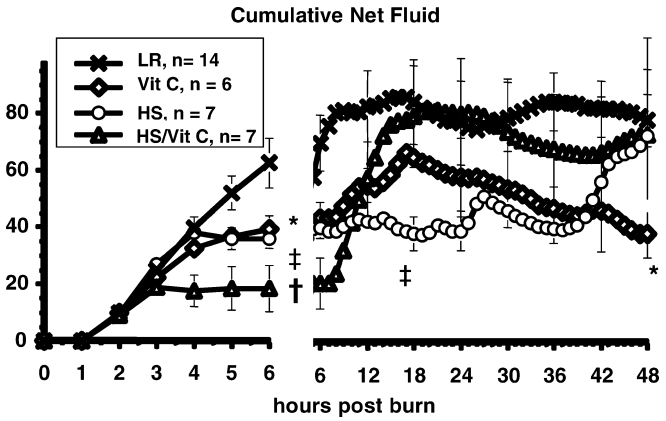

FIG. 4. **Cumulative net fluid balance defined as fluid infused less urine output during the first 6 h after burn and from 6 to 48 h after thermal injury**. Data expressed as mean ± SEM. *Vitamin C versus LR, †HS/vitamin C versus LR, ‡HS versus LR at $P < 0.05$.

burn and resuscitation. Right atrial pressure and pulmonary arterial occlusion pressure decreased only slightly, 1 to 2 mmHg, after burn injury and were then restored to near normal values.

In accordance with the resuscitation protocol, which adjusted infusion to maintain urine output between 1 and 2 mL/kg, urine outputs were similar in all groups (Fig. 3). However, both HS groups exhibited an early diuresis of 3 to 4 mL/kg/h that resolved after 4 to 5 h. Vitamin C infusion significantly reduced fluid requirements and, therefore, net fluid balance (fluid in, urine out) by about 30% at 6 h in comparison with the LR group, and at 48 h, the fluid infused and net fluid balance were reduced from 159 ± 10 mL/kg to 126 ± 8 mL/kg and 65 ± 11 mL/kg to 34 ± 8 mL/kg, respectively, in comparison with the LR group ($P < 0.05$; Fig. 4). HS and HS/vitamin C infusion reduced fluid requirements by 30% and 65%, respectively, at 6 h, but the volume-sparing effect of HS was not observed after 36 h and that of HS/vitamin C was lost after 12 h (Fig. 4).

Plasma total antioxidant potential, as an indicator of vitamin C levels, increased about 25-fold ($P < 0.05$) at 2 and 3 h in response to vitamin C infusion compared with the LR and HS groups and remained about 5 to 10-fold higher throughout the rest of the study (Fig. 5). Vitamin C infusion also prevented the 4-fold increase in plasma TBARS seen in the LR group early after burn ($P < 0.05$; Fig. 6).

In heart, lung, liver, ileum, and skeletal muscle, total antioxidant potential was similar in both vitamin C groups compared with the LR group (Table 1). Indices of lipid peroxidation (Table 1) or activities of antioxidant enzymes (superoxide dismutase, glutathione reductase, and glutathione peroxidase; Table 2) were not significantly different among the groups in any of the tissues examined. Also, no significant differences in tissue reduced or oxidized glutathione concentrations were observed among groups (Table 1).

DISCUSSION

The hemodynamic response to burn injury and resuscitation was similar to what we have reported previously, i.e., a reduction in cardiac output with little effect on mean arterial pressure (29, 30). In this sheep burn model, substantial early volume sparing was achieved with vitamin C, HS, or the combination, while maintaining mean arterial pressure and cardiac output as effectively as standard-of-care LR treatment. However, only isotonic vitamin C maintained this volume-sparing effect at 48 h after burn. In general, these studies support previous investigations with large doses of vitamin C in a guinea pig burn model and preliminary studies in burn patients. For example, it was reported in thermally injured

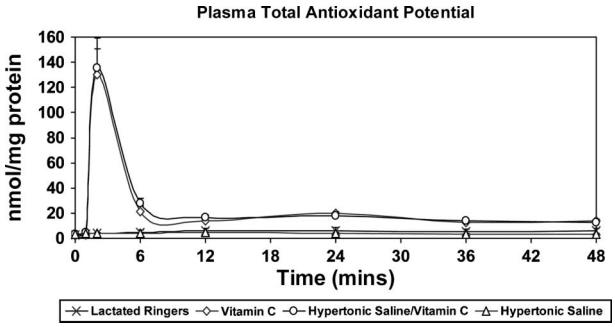

FIG. 5. **Plasma total antioxidant potential over the 48-h experimental period after burn injury**. Data expressed as mean ± SEM. Values in the vitamin C and HS/vitamin C groups are significantly higher ($P < 0.05$) from the LR group for up to 24 h after thermal injury.

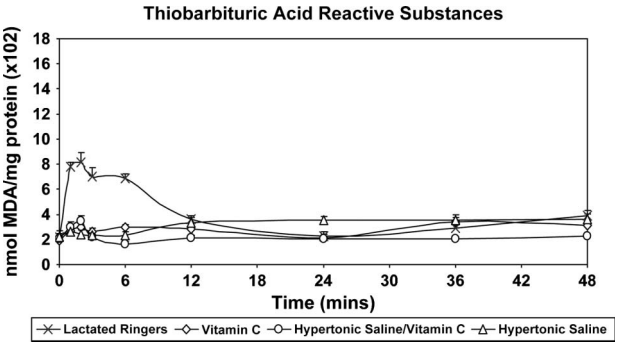

FIG. 6. **Plasma TBARS over the 48-h experimental period after burn injury**. Data expressed as mean ± SEM. TBARS levels in the LR group are significantly higher ($P < 0.05$) than the levels in the other groups for the first 6 h after thermal injury.

guinea pigs that infusion of 14 mg/kg/h vitamin C reduced the 24-h fluid requirements for LR from 4 mL/kg/percentage of burn to 1 mL/kg/percentage of burn while maintaining comparable cardiac outputs (19, 24). In humans, high-dose vitamin C reduced fluid requirements to 60% of that in the LR-treated group, reduced burn tissue water content 50%, and reduced ventilator days, while hemodynamic variables and base deficits were similar in both groups (35). However, a small study using lower doses failed to observe a volume-sparing effect of vitamin C in the treatment of burn patients (36). In addition, in the present study, high-dose vitamin C treatment markedly increased plasma total antioxidant potential and reduced indices of plasma lipid peroxidation in the first 6 h after burn. At the tissue level, vitamin C maintained overall antioxidant status in major organs.

At present, it is unknown why the volume-sparing effects of HS were not maintained in the presence of high-dose vitamin C. HS was shown to reduce fluid volume and improve tissue perfusion early after burn injury (29, 37). This observation was further supported by a recent meta analysis by Milner et al. (38), which examined 10 published studies of 2% to 3% NaCl with control groups that included a total of 536 patients. They reported that HS reduced 24-h fluid volume requirements about one-third and the effects persisted for 48 h. Previous animal studies in our laboratory also showed an early volume-sparing effect of 7.5% HS/Dextran (HSD). However, we have typically observed a rebound of fluid requirements and net fluid accumulation beginning 8 to 12 h after an initial treatment with 4 mL/kg HSD. When a second dose of HSD was administered after the volume-sparing benefit had begun to wane, the volume-sparing effect was sustained up to 48 h after the burn injury (29, 30). Thus, it was noted that the volume-expanding effects of this hypertonic fluid may depend not only on the dose, but also on the dosing interval and infusion rate.

The exact mechanisms through which such high doses of vitamin C act to reduce fluid requirements remain unknown, although it has been speculated that the vitamin C reduces the increased capillary permeability and large increase in negative interstitial pressure developing in tissue after burn through its antioxidant properties (20, 39). However, it is unknown whether other mechanisms are involved. It should also be noted that the addition of 10 g of vitamin C to 500 mL of LR results in a mildly hypertonic mixture, 386 mOsm/L, this is not much different than the osmolality of normal saline, which is 308 mOsm/L, and much less than the 1000 mOsm/L of the hypertonic fluids used in the present study (equivalent to 3% saline). Thus, it appears that the beneficial effects of vitamin C cannot be explained solely on its osmolality. It has been suggested that the capillary leak that develops is sealed within 24 to 36 h after burn (40), whereas others state that it can seal as early as 6 to 12 h after burn (41). Therefore, additional studies into the effects of vitamin C on these mechanisms will need to determine whether the current data are consistent with these time frames. In addition, such studies may also provide insight into the proper resuscitation regimen necessary if vitamin C is to be added to hypertonic fluids to reduce fluid requirements in the resuscitation from thermal injury.

The overall safety of such high doses of vitamin C for the treatment of burns remains unknown. Studies indicate that vitamin C levels in plasma respond to intravenous doses. For example, in humans treated with 1 g/h for 18 h, plasma vitamin C levels were raised 14- to 30-fold in comparison with patients who did not receive vitamin C (36). It was also reported that high-dose ascorbic acid infusion resulted in peak plasma vitamin C levels near 540 μmol/L; i.e., about 20-fold higher than normal plasma vitamin C concentrations (35). These levels peaked 12 to 18 h after start of infusion and returned to baseline about 48 h after the end of infusion. It is not known to what extent such doses affect the vitamin C content of tissues. The present study is the first to examine such high doses of vitamin C on antioxidant status in multiple tissues. As indicated, antioxidant status of the tissues examined was maintained in vitamin C-treated animals and no evidence for a pro-oxidant effect was noted. This is particularly of note because it could be hypothesized that vitamin C could react with free iron that is liberated from the hemolysis of blood and the increase in free myoglobin from injured muscle that occurs after burn, and induce further generation of ROS. Tanaka et al. (35) also observed less lipid peroxidation in plasma from burn patients treated with 66 mg/kg/h vitamin C compared with LR-treated patients. In addition, humans treated with 33 mg/kg/h vitamin C showed no abnormalities in blood cell counts, or liver or kidney function for 7 d after vitamin C treatment (42). It should also be noted that i.v. infusion of 750 to 7500 mg of vitamin C daily for 6 d to healthy volunteers also did not induce a pro-oxidant effect in plasma biomarkers (43).

TABLE 1. **Effect of high dose vitamin C on tissue total antioxidant status***

	Antioxidant potential					TBARS				
	Heart	Lung	Liver	Muscle	Ileum	Heart	Lung	Liver	Muscle	Ileum
LR	138 ± 5	90 ± 8	86 ± 13	18 ± 2	32 ± 2	0.49 ± 0.06	1.01 ± 0.12	1.00 ± 0.12	0.17 ± 0.03	1.48 ± 0.35
Vitamin C	146 ± 9	103 ± 5	100 ± 7	20 ± 2	35 ± 1	0.41 ± 0.02	0.66 ± 0.05	0.66 ± 0.05	0.24 ± 0.02	1.44 ± 0.12
HS	125 ± 8	109 ± 9	85 ± 4	15 ± 1	32 ± 1	0.62 ± 0.14	0.96 ± 0.13	0.96 ± 0.13	0.27 ± 0.03	1.23 ± 0.13
HS/Vitamin C	153 ± 14	113 ± 8	114 ± 13	19 ± 2	65 ± 1	0.46 ± 0.05	0.87 ± 0.10	0.87 ± 0.10	0.27 ± 0.03	1.57 ± 0.25
	Reduced glutathione					Oxidized glutathione				
	Heart	Lung	Liver	Muscle	Ileum	Heart	Lung	Liver	Muscle	Ileum
LR	32.1 ± 2.4	3.8 ± 0.8	28.3 ± 4.3	3.66 ± 0.34	41.3 ± 5.34	0.94 ± 0.11	0.15 ± 0.02	1.83 ± 0.19	0.93 ± 0.26	1.78 ± 0.20
Vitamin C	37.5 ± 1.5	4.1 ± 0.2	28.9 ± 1.3	6.45 ± 0.41	42 ± 3.0	0.94 ± 0.07	0.23 ± 0.04	1.00 ± 0.12	0.36 ± 0.02	1.06 ± 0.09
HS	36.2 ± 2.8	6.2 ± 0.6	31.1 ± 4.8	5.08 ± 0.67	34.8 ± 5.04	0.70 ± 0.07	0.27 ± 0.05	2.24 ± 0.45	0.39 ± 0.01	1.31 ± 0.27
HS/Vitamin C	34.3 ± 1.6	4.5 ± 0.9	24.4 ± 2.9	4.51 ± 0.35	33.5 ± 2.49	1.04 ± 0.34	0.17 ± 0.03	2.25 ± 0.48	0.67 ± 0.09	1.98 ± 0.34

*Data expressed as mean ± SE of nanomoles per milligram of protein for five to seven animals per group.

TABLE 2. **Effect of high dose vitamin C on tissue antioxidant enzyme activities***

	Glutathione peroxidase					Glutathione reductase ($\times 10^{2}$)				
	Heart	Lung	Liver	Muscle	Ileum	Heart	Lung	Liver	Muscle	Ileum
LR	0.19 ± 0.01	0.06 ± 0.01	0.16 ± 0.01	0.02 ± 0.001	0.12 ± 0.01	1.56 ± 0.29	1.4 ± 0.2	2.85 ± 0.46	3.55 ± 0.17	4.02 ± 0.29
Vitamin C	0.20 ± 0.01	0.05 ± 0.01	0.10 ± 0.01	ND[4]	0.12 ± 0.01	1.50 ± 0.13	1.4 ± 0.1	2.04 ± 0.12	ND[4]	3.9 ± 0.3
HS	0.22 ± 0.02	0.05 ± 0.01	0.15 ± 0.02	0.02 ± 0.003	0.13 ± 0.01	1.86 ± 0.14	1.4 ± 0.3	2.12 ± 0.57	3.90 ± 0.68	3.65 ± 0.47
HS/Vitamin C	0.21 ± 0.01	0.06 ± 0.004	0.21 ± 0.03	0.03 ± 0.003	0.12 ± 0.02	3.34 ± 0.15	1.3 ± 0.2	3.28 ± 0.79	3.61 ± 0.44	4.28 ± 0.24
	Cu-Zn SOD†					MnSOD‡				
	Heart	Lung	Liver	Muscle	Ileum	Heart	Lung	Liver	Muscle	Ileum
LR	3.2 ± 0.6	1.0 ± 0.2	19.3 ± 1.9	1.0 ± 0.1	3.0 ± 0.4	7.1 ± 0.5	1.2 ± 0.1	7.6 ± 0.7	1.2 ± 0.1	1.7 ± 0.3
Vitamin C	3.8 ± 0.2	1.1 ± 0.1	20.8 ± 1.2	ND§	2.8 ± 0.2	6.1 ± 0.2	1.9 ± 0.1	7.2 ± 0.2	ND[4]	2.7 ± 0.2
HS	2.9 ± 1.0	1.3 ± 0.2	19.4 ± 2.2	0.9 ± 0.2	2.0 ± 0.3	8.3 ± 1.5	1.8 ± 0.1	7.9 ± 0.7	1.2 ± 0.2	2.2 ± 0.3
HS/Vitamin C	2.9 ± 0.2	1.3 ± 0.3	16.4 ± 1.9	1.0 ± 0.1	2.3 ± 0.4	8.2 ± 0.4	1.9 ± 0.2	8.4 ± 0.7	1.3 ± 0.2	2.8 ± 0.3
	Catalase									
	Heart	Lung	Liver	Muscle	Ileum					
LR	42.2 ± 5.67	49.4 ± 5.71	578 ± 71	6.6 ± 1.03	36.4 ± 8.99					
Vitamin C	45 ± 6	58.2 ± 6.8	533 ± 21	4.6 ± 0.2	34.7 ± 3.1					
HS	46.7 ± 5.14	54.5 ± 5.35	656 ± 110	8.6 ± 2.51	37.0 ± 2.55					
HS/Vitamin C	54.3 ± 10.64	59.3 ± 4.88	530 ± 58	7.6 ± 1.13	41.5 ± 3.08					

*Data expressed as units per milligram of protein.
†Copper zinc superoxide dismutase.
‡Manganese superoxide dismutase.
§ND, not determined.

In general, very few studies have examined the toxicity of such high doses of vitamin C. Single oral doses of 4 g of ascorbic acid increased uric acid excretion and acidification of urine, and oxalate excretion was unchanged (44). Schmidt et al. (45) reported that a daily dose of 9 g was tolerated in humans before an increase in urinary oxalate, the primary endpoint of the study, was observed. Others have reported that doses as high as 5 to 10 g/day for more than 3 years were well tolerated (46). In an anecdotal report, an individual appeared to tolerate intravenous doses of 40 g of ascorbic acid three times a week that was supplemented with 20 to 40 g/day orally (47). However, hemolysis of blood was observed when the intravenous dose was increased to 80 g. In general, mild gastrointestinal disturbances have been reported in individuals consuming 1 to 4 g/day (44), which has led a recent report by the Institute of Medicine (48) to recommend 2 g of vitamin C as a safe upper limit for chronic daily ingestion. However, recommendations for vitamin C for chronic use may have little relevance to its acute use in the present study for the treatment of severe burn injury. In addition, whereas plasma concentrations of vitamin C are tightly regulated after oral consumption, plasma levels after i.v. administration continue to rise in a dose-dependent manner (49).

In conclusion, the present data are encouraging that addition of antioxidants such as vitamin C may help reduce the requirements for large volumes of crystalloid fluids for the resuscitation of burn shock, are safe, and in situations where logistic constraints limit fluid availability, early volume-sparing effects may be life saving. Other potentially important benefits for military use were shown by Sakurai et al. (26) who observed that vitamin C treatment delayed 6 h after burn was still effective in reducing burn wound edema and maintaining hemodynamic stability in comparison with LR-treated animals. Others have shown that infusion of vitamin C for up to 8 h after thermal injury was as effective as 24-h treatment in reducing total fluid requirements for burn resuscitation (21). Although the limited human studies to date have not significantly affected

outcome, additional research in this area appears warranted as efforts continue to identify small-volume fluid resuscitation strategies for the treatment of thermal injuries.

ACKNOWLEDGMENTS

This study adhered to National Institutes of Health guidelines for the care and use of laboratory animals (DHHS Publication, NIH, 86-23). The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense (AR 360-5). The authors thank Amber Large for assistance in preparation of the manuscript.

REFERENCES

1. Monafo WW: Initial management of burns. *N Engl J Med* 335:1581 1586, 1996.
2. Jordan BS, Harrington DT: Management of the burn wound. *Nurs Clin North Am* 32:251 273, 1997.
3. Pruitt BA Jr, Goodwin CW, Mason AD Jr: Epidemiological, demographic, and outcome characteristics of burn injury. In Herndon DN (ed): *Total Burn Care*, Ed 2. Philadelphia: WB Saunders Company, 2002, pp 16 30.
4. Anonymous: Proceedings of the 15th Conference on Military Medicine Uniformed Services University of the Health Sciences. *Milit Med* 167(Suppl 2): 1 29, 2002.
5. Cancio LC, Mozingo DW, Pruitt BA Jr: The technique of fluid resuscitation for patients with severe thermal injuries. *J Crit Illn* 12:183 190, 1997.
6. Griswold JA, Anglin BL, Love RT Jr: Hypertonic saline resuscitation: efficacy in a community-based burn unit. *South Med J* 84:692 696, 1991.
7. Shirani KZ, Vaughan GM, Mason AD Jr, Pruitt BA Jr: Update on current therapeutic approaches in burns. *Shock* 5:4 16, 1996.
8. Warden GD: Fluid resuscitation and early management. In Herndon DN (ed): *Total Burn Care*, Ed 2. Philadelphia: WB Saunders Company, 1996, pp 53 60.
9. Williams WG, Phillips LG: Pathophysiology of the burn wound. In Herndon DN (ed): *Total Burn Care*, Ed 2. Philadelphia: WB Saunders Company, 1996, pp 63 70.
10. Till GO, Hatherill JR, Tourtellotte WW, Lutz MJ, Ward PA: Lipid peroxidation and acute lung injury after thermal trauma to skin. *Am J Pathol* 119:376 384, 1985.
11. Youn Y-K, Lalonde C, Demling R: Oxidants and the pathophysiology of burn and smoke inhalation injury. *Free Radical Biol Med* 12:409 415, 1992.
12. Nguyen TT, Cox CS, Traber DL, Gasser H, Redl H, Schlag G, Herndon DN: Free radical activity and loss of plasma antioxidants, vitamin E, and sulfhydryl groups in patients with burns: the 1993 Moyer Award. *J Burn Care Rehabil* 14:602 609, 1993.
13. Leff JA, Burton LK, Berger EM, Anderson BO, Wilke CP, Repine JE: Increased serum catalase activity in rats subjected to thermal skin injury. *Inflammation* 17:199 204, 1993.
14. Cetinkale O, Belce A, Konukoglu D, Senyuva C, Gumustas M, Tas T: Evaluation of lipid peroxidation and total antioxidant status in plasma of rats following thermal injury. *Burns* 23:114 116, 1997.
15. Hatherill JR, Till GO, Bruner LH, Ward PA: Thermal injury, intravascular hemolysis, and toxic oxygen products. *J Clin Invest* 78:629 636, 1986.
16. Till GO, Beauchamp C, Menapace D, Tourtellotte W Jr, Kunkel R, Johnson KJ, Ward PA: Oxygen radical dependent lung damage following thermal injury of rat skin. *J Trauma* 23:269 277, 1983.
17. Wan HL, Tsai PJ, Chiang MJ, Wu JP, Lui L, Tsai WJ, Kuo J-S, Yang C-S: Effect of acute skin thermal injury on subcutaneous glutathione, ascorbic acid and hydroxyl radical concentrations in anesthetized rats. *Redox Rep* 2:267 272, 1996.
18. Rock CL, Dechert RE, Khilnani R, Parker RS, Rodriguez JL: Carotenoids and antioxidant vitamins in patients after burn injury. *J Burn Care Rehabil* 18:269 278, 1997.
19. Matsuda T, Tanaka H, Williams S, Hanumadass M, Abcarian H, Reyes H: Reduced fluid volume requirement for resuscitation of third-degree burns with high-dose vitamin C. *J Burn Care Rehabil* 12:525 532, 1991.
20. Matsuda T, Tanaka H, Shimazaki S, Matsuda H, Abcarian H, Reyes H, Hanumadass M: High-dose vitamin C therapy for extensive deep dermal burns. *Burns* 18:127 131, 1992.
21. Matsuda T, Tanaka H, Reyes HM, Richter HM, Hanumadass MM, Shimazaki S, Matsuda H, Nyhus LM: Antioxidant therapy using high-dose vitamin C: reduction of postburn resuscitation fluid volume requirements. *World J Surg* 19:287 291, 1995.
22. Tanaka H, Broaderick P, Shimazaki S, Matsuda H, Hansalia H, Hanumadass M, Reyes H, Matsuda T: How long do we need to give antioxidant therapy during resuscitation when its administration is delayed for two hours. *J Care Rehab* 13:567 572, 1992.
23. Tanaka H, Hanumadass M, Matsuda H, Shimazaki S, Walter RJ, Matsuda T: Hemodynamic effects of delayed initiation of antioxidant therapy (beginning two hours after burn) in extensive third-degree burns. *J Burn Care Rehabil* 16:610 615, 1995.
24. Tanaka H, Matsuda H, Shimazaki S, Hanumadass M, Matsuda T: Reduced resuscitation fluid volume for second-degree burns with delayed initiation of ascorbic acid therapy. *Arch Surg* 132:158 161, 1997.
25. Tanaka H, Shimizu S, Tokunaga T, Matsuda T, Shimazaki S: High-dose vitamin C prevents acute lung injury due to lung lipid peroxidation after severe thermal injury. *J Burn Care Rehabil* 20:S139, 1999.
26. Sakurai M, Tanaka H, Matsuda T, Goya T, Shimazaki S, Matsuda H: Reduced resuscitation fluid volume for second-degree experimental burns with delayed initiation of vitamin C therapy (beginning 6 h after injury). *J Surg Res* 73:24 27, 1997.
27. Smith JG, Kramer GC, Perron P, Nakayama S-I, Gunther RA, Holcroft JW: A comparison of several hypertonic solutions for resuscitation of bled sheep. *J Surg Res* 39:517 528, 1985.
28. Wade CE, Hannon JP, Bossone CA, Hunt MM, Loveday JA, Coppes RI, Gildengorin VL: Resuscitation of conscious pigs following hemorrhage. Comparative efficacy of small volume resuscitation. *Circ Shock* 29:193 204, 1989.
29. Elgjo GI, Mathew BP, Poli de Figueiredo LF, Schenarts PJ, Horton JW, Dubick MA, Kramer GC: Resuscitation with hypertonic saline Dextran improves cardiac function *in vivo* and *ex vivo* after burn injury in sheep. *Shock* 9:375 383, 1998.
30. Elgjo GI, Traber DL, Hawkins HK, Kramer GC: Burn resuscitation with two doses of 4 mL/kg hypertonic saline Dextran provides sustained fluid sparing: a 48-hour prospective study in conscious sheep. *J Trauma* 49:251 263, 2000.
31. Benzie IFF, Strain JJ: Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol* 299:15 27, 1999.
32. Naito C, Kawamura M, Yamamato Y: Lipid peroxides as the initiating factor of atherosclerosis. *Ann N Y Acad Sci* 676:27 45, 1993.
33. Dubick MA, Carden SC, Jordan BS, Langlinais PC, Mozingo DW: Indices of antioxidant status in rats subjected to wood smoke inhalation and/or thermal injury. *Toxicology* 176:145 157, 2002.
34. Anderson ME: Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol* 113:548 555, 1985.
35. Tanaka H, Matsuda T, Miyagantani Y, Yukioka T, Matsuda H, Shimazaki S: Reduction of resuscitation fluid volumes in severely burned patients using ascorbic administration. *Arch Surg* 135:326 331, 2000.
36. Mann R, Foster K, Kemalyan N, Gibran NS, Engrav LH, Heimbach DM: Intravenous vitamin C in clinical burn resuscitation. *J Burn Care Rehab* 18:S87, 1997.
37. Kien ND, Antognini JF, Reilly DA, Moore PG: Small-volume resuscitation using hypertonic saline improves organ perfusion in burned rats. *Anesth Analg* 83:782 788, 1996.
38. Milner SM, Thomas JA, Grady JJ, Herndon DN, Phillips LG, Kramer GC: The clinical record of hypertonic saline in burn resuscitation: a meta-analysis of controlled clinical studies. *J Burn Care Rehab* 18:S88, 1997.
39. Matsuda T, Tanaka H, Yuasa H, Forrest R, Matsuda H, Hanumadass M, Reyes H: The effect high-dose vitamin C therapy on postburn lipid peroxidation. *J Burn Care Rehabil* 14:624 629, 1993.
40. Baxter CR: Fluid volume and electrolyte changes of the early postburn period. *Clin Plastic Surg* 1:693 709, 1974.
41. Aikawa N, Ishibiki K, Naito C, Abe O, Yamamoto S, Motegi M, Sudo M: Individualized fluid resuscitation based on extensive burns. *Burns* 8:249 255, 1982.
42. Matsuda T, Yuasa H, Khabbaz W, Yoshitake M, Ahmad N, Reyes H: Study of the safety of continuous intravenous infusion of high dose-vitamin C in healthy human volunteers. *Proc Am Burn Assoc* 26:141, 1994.
43. Mühlhöfer A, Mrosek S, Schlegel B, Trommer W, Rozario F, Böhles H, Schremmer D, Zoller WG, Biesalski HK: High-dose intravenous vitamin C is not associated with an increase of pro-oxidative biomarkers. *Eur J Clin Nutr* 58:1151 1158, 2004.
44. Meyers DG, Maloley PA, Weeks D: Safety of antioxidant vitamins. *Arch Intern Med* 156:925 935, 1996.
45. Schmidt K-H, Hagmaier V, Hornig DH, Vuilleumier J-P, Rutishauser G: Urinary oxalate excretion after large intakes of ascorbic acid in man. *Am J Clin Nutr* 34:305 311, 1981.
46. Bendich A, Langseth L: The health effects of vitamin C supplementation: a review. *J Am Coll Nutr* 14:124 136, 1995.
47. Rees DC, Kelsey H, Richards JDM: Acute haemolysis by high dose ascorbic acid in glucose-6- phosphate deficiency. *Br Med J* 306:841 842, 1993.
48. Institute of Medicine of the National Academy of Sciences: *Dietary Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*. Washington, DC: National Academy Press, 2000.
49. Padayatty SJ, Sun H, Wang Y, Riodan HD, Hewitt SM, Katz R, Wesley RA, Levine M: Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann Intern Med* 140:533 537, 2004.