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RESUSCITATION WITH HYPERTONIC SALINE DEXTRAN IMPROVES CARDIAC FUNCTION *IN VIVO* AND *EX VIVO* AFTER BURN INJURY IN SHEEP

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ABSTRACT—In a 24 h, double-blind, prospective trial, we tested the hypothesis that two 4 mL/kg doses of hypertonic saline dextran (HSD; 7.5% NaCl/6% dextran 70) given in addition to isotonic fluid treatment would produce both immediate and sustained benefit for the heart after large burn injury. 12 instrumented sheep were subjected to a 40% total body surface area full-thickness flame burn under halothane anesthesia. 1 h after burn, when the animals had recovered from anesthesia, the first dose of either HSD (n = 6) or normal saline (NaCl .9%; n = 6) was infused over 30 min. The test solution was immediately followed by lactated Ringer's solution infused to maintain a urine output of 1–2 mL/kg·h throughout the study. The second dose of test solution was started at 12 h and was infused over 5 h. The initial dose of HSD corrected the burn-induced reduction in cardiac output, cardiac work, an index of myocardial contractility, and restored myocardial blood flow, as measured by the colored microsphere technique, to preburn values. Plasma concentrations of troponin I, creatine kinase (CK), and CK isoenzyme CKMB were increased 1 h after burn, but were not altered after HSD treatment. After euthanasia at 24 h, myocardial glutathione concentrations were higher in HSD-treated animals, whereas other markers of oxidative injury in heart or in plasma did not show systematic differences. The maximum contraction force measured in isolated right papillary muscles *ex vivo* was significantly greater in HSD-treated than normal saline-treated animals. In conclusion, the first dose of 4 mL/kg HSD infused 1 h after burn improved cardiac function, whereas the second dose of HSD infused at 12 h was without apparent effect on dynamic variables. An overall effect of the HSD treatments was a lasting increase in papillary muscle contraction force.

INTRODUCTION

Large burn injuries are associated with compromised cardiovascular function and depressed myocardial contractility (1); the contractile deficit cannot be overcome by excess glucose, insulin, coronary flow, or maximal preload elevation (2). Initial treatment using small volumes of hypertonic saline dextran (HSD) preceding resuscitation with lactated Ringer's solution (LR) has been shown to improve myocardial contractility in isolated hearts from burn-injured guinea pigs after 24 h (3). Transiently reduced afterload and augmented preload of about 10 min duration have been recorded after intravenous HSD injection in anesthetized, thermally injured dogs, but without direct evidence of improved cardiac contractility (4). In contrast, improved cardiac contractile performance with HSD has been shown after ischemia-reperfusion in isolated rat hearts (5). In the only clinical trial to date, evidence for benefit to the heart was observed as decreased plasma levels of the cardiac markers troponin I and creatine kinase (CK) when HSD was given within 6 h of burn injury (6).

In a 24 h prospective, double-blind study in a conscious sheep model, we tested the hypothesis that the cardioprotective effect of a single, initial dose of HSD, followed by standard LR resuscitation, would be enhanced by a second dose of HSD administered 12 h after burn. The cardiac effects of HSD treatment were compared against a group of animals given two test doses of an equal volume of normal saline (NS; NaCl .9%). Because burn injury is associated with the generation of free radicals (7, 8), we also examined evidence for lipid peroxidation and antioxidant status in the heart and in plasma after burn injury.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Animal Care and Use Committee of the University of Texas Medical Branch at Galveston, with adherence to National Institutes of Health guidelines for care and use of laboratory animals (Department of Health and Human Services Publication, National Institutes of Health 86-23).

Preparatory surgery

Adult female Merino sheep (n = 12) weighing 26–42 kg were orotracheally intubated, mechanically ventilated, and surgically prepared under halothane anesthesia in a sterile operating environment. Indwelling vascular catheters were placed in the following vessels: 1) abdominal aorta through the right femoral artery for blood pressure and heart rate (HR) monitoring and recording and for arterial blood sampling, and 2) the inferior vena cava via the right femoral vein for maintenance fluid infusion during surgery and for test fluid and LR administration after burn. A 7 French (Fr) flow-directed thermodilution pulmonary artery catheter (Swan-Ganz 131-7F, Baxter Healthcare Corp., Ir-

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vine, CA) was introduced through the right jugular vein and the tip placed in the pulmonary artery. This catheter was used for measurement of pulmonary arterial and capillary wedge pressure and cardiac output by thermodilution and for sampling mixed venous blood for blood gas analysis. Through a left thoracotomy in the fifth intercostal space, a Silastic catheter (Dow Corning, .025 in. inner diameter) was positioned with the tip in the left atrium, and the other end exteriorized through a para-incisional opening. This catheter was used for left atrial pressure (LAP) measurements and for colored microsphere injections. Catheters were filled with heparin sodium solution and secured to the fleece of the back of the animal. The sheep were allowed 5–7 days recovery in the animal intensive care unit before the experimental burn.

On the day before burn, the vascular catheters were attached to pressure transducers (Baxter Pressure Monitoring Kit, Baxter Healthcare, Irvine, CA) connected to an HP Patient Monitor (model 78901A, Hewlett-Packard, Andover, MA) for continuous hemodynamic monitoring and to condition the sheep to the experimental situation. Beginning 24 h before burn, the sheep were denied free access to water but were allowed food. Isotonic saline was infused at 15 mL/h to keep the intravenous catheters patent.

Experimental protocol

Baseline recordings for hemodynamic parameters and blood tests were obtained in the conscious animal on the morning of the experiment. Under orotracheal intubation, deep halothane anesthesia, and mechanical ventilation, the sides of the sheep were sheared, and the 40% total body surface area (TBSA) to be burned was outlined on the skin with a marker. At this time, a 12 Fr Foley urinary catheter was inserted in the bladder, secured to the tail area, and connected to a commercial urine collection bag. Each subsequent hour, the bag was emptied into a graduated cylinder and the volume recorded. A full-thickness burn, covering the sides and back of the animal, was produced with a Bunsen burner. The flame was carefully shielded to create a sharp transition zone between burned and unburned skin, and adequate burn depth was assessed by blanching and contraction of the skin. A full-thickness burn leaves an insensible area, but the thin margin of partial thickness burn encompassing the full-thickness lesion may give rise to discomfort. If the animal did not show alertness and interest in the surroundings within a few hours after recovering from the anesthesia, an injection of buprenorphine 3 mg was given intramuscularly. Animals were not allowed access to food and water for the remaining 24 h of the experiment.

1 h after burn, with animals fully recovered from anesthesia, a 4 mL/kg dose of either HSD or NS was infused over 30 min. This treatment was immediately followed by continuous infusion of LR, with the infusion rate adjusted each hour to produce a urine output of 1–2 mL/kg·h until the end of the experiment. 12 h after burn injury, the second 4 mL/kg dose of HSD or NS was started, and was given over 5 h, simultaneously with the continuous LR infusion.

The starting time for the second infusion was based on the assumption that the volume-expanding effect of the first dose would have almost disappeared 12 h after burn, which corresponds to approximately 1.2 half-lives of HSD in plasma (9). The slow infusion rate was based on the assumption that after stabilization of cardiovascular function, a slower rate would be sufficient for HSD to exert a clinically noticeable effect. The animals were assigned to the protocol so that a particular test solution would not be used in more than two animals in a row, and both regimens would be used in six animals each by completion of the 12 experiments. The investigators performing the experiment were blinded to the assignment technique and to the test solutions, which were prepared and delivered in coded bags.

24 h after burn, sheep were deeply anesthetized with halothane and intubated; the heart was removed, and a section of the right ventricular wall including papillary muscle was immediately transferred to aerated Krebs solution for measurements of contraction force. During autopsy, tissue samples from the heart were taken from the right ventricular wall and from the left epi- and endocardium for myocardial blood flow (MBF) estimation, tissue water weight to dry weight ratio calculation, and oxidative injury assays. Myocardial tissue from five unburned animals was collected and used as controls for edema and oxidative injury analysis and for studying myocardium maximum contraction force in papillary muscle.

Experimental measurements

Hemodynamic measurements—The following parameters were monitored continuously and their values recorded at fixed time points: mean arterial pressure (MAP, mmHg), central venous pressure (CVP, mmHg), pulmonary arterial pressure (PAP, mmHg), LAP (mmHg), and HR (beats per min). Cardiac outputs (L/min) were measured every hour. Cardiac function was assessed by calculation of cardiac index (CI, L/min·m^2), stroke volume index (SVI, L/stroke·m^2), left and right ventricular stroke work indexes (LVSWI and RVSWI, Joule/stroke·m^2), and left and right cardiac work indexes (LCWI and RCWI, Joule/min · m^2). Systemic and pulmonary vascular resistance index (dyn·s/cm^5·m^2), oxygen delivery (mL/min·m^2), oxygen consumption (mL/min·m^2), and oxygen extraction rate (%) were calculated using standard equations (10).

Blood and urine analyses—Arterial blood gases, acid/base balance, and hemoglobin were measured on an ILS Blood Gas, Electrolyte, and 482 CO-Oximeter (Instrument Laboratory Systems, Lexington, MA), and blood from the same sample was centrifuged for hematocrit (HCT). Plasma and urine Na^+, K^+, and Cl^- were measured with a Lablyte System 810 plasma ion electrolyte analyzer (Beckman, Brea, CA). Analyses for troponin I (ng/mL) and CK subunit MB (CKMB, ng/mL) were performed on a Stratus II Immunoassay system (Baxter Healthcare Corp., Chicago, IL) and for total CK (IU/g plasma protein) on a Vitris System 750 XRC (Johnson & Johnson). Alanine aminotransferase (ALT, IU/g plasma protein) and aspartate aminotransferase (AST, IU/g plasma protein) were analyzed on a Hitachi 911 Clinical Chemistry Analyzer (Boehringer Mannheim, Indianapolis, IN). Plasma was also analyzed for thiobarbituric acid-reactive substances (TBARS, nmol malondialdehyde (MDA)/g plasma protein) as described in the following paragraph.

Tissue analyses for oxidative injury—Cardiac tissue collected during autopsy for assessment of antioxidant status was stored at −70°C until analyzed. All analyses were completed within 3 months of collection. Ventricular wall tissue was homogenized in 50 mM potassium phosphate buffer, pH 7.4. TBARS were analyzed in the butanol phase according to the procedure described by Naito et al. (11). Superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) activities were determined spectrophotometrically (Beckman DU 70 Spectrophotometer) as previously described (12). Reduced glutathione was determined enzymatically as described by Anderson (13).

MBF—Fluorescent polyvinyl microspheres (NuFlow®, Interactive Medical Technologies Ltd., Los Angeles, CA) were used to determine MBF. Approximately $5{\cdot}10^6$ spheres of mean diameter 15.5 μm were injected as a 10 s bolus into the left atrium at baseline, 2 h, 12 h, and 24 h after burn, using a different fluorescent color at each time point. Arterial blood was withdrawn from the femoral arterial catheter at a constant rate of 8 mL/min starting 10 s before and continuing for 2 min after the injection, for determination of the reference arterial calibration constant used in tissue perfusion calculation. Microspheres are distributed to all tissues and lodge in the microvasculature. Blood flow values were calculated according to the following equation: $Q_m = (C_m \cdot Q_r)/C_r$ where Q_m is the tissue flow per g (mL/min·g), C_m is the microsphere count per g tissue, Q_r is the withdrawal rate of the reference blood sample (mL/min), and C_r is the microsphere count in the reference blood sample (14). At autopsy, tissue samples were taken from the right ventricle (full wall thickness) and from the left ventricle epi- and endocardium. The samples were sent to Interactive Medical Technologies, where microspheres were recovered by tissue digestion and extraction and counted by flow cytometry techniques for calculation of MBF.

Myocardial maximum contraction force—A small piece of right ventricular papillary muscle was isolated immediately after removal of the beating heart, suspended in aerated Krebs solution, and maintained at 36.6 ± .5°C at a constant pH of 7.42 and an atmospheric pressure of ≥ 500 mmHg O_2. The medium was changed every 5 min. Papillary muscle from the right ventricle was chosen, as LV papillary muscle diameter was considered too large to ensure proper central oxygenation of the muscle in the tissue chamber. One end of the muscle was secured at the bottom of the tissue bath with a spring-loaded clip; the other end was connected to a Grass FT03 force transducer. After a 2 h stabilization period, the muscle was gradually stretched during continuous supramaximal electrical stimulation at .2 Hz (12 beats per min), delivered from a Grass S88 stimulator, until maximum contraction force was obtained (Lmax, g) (15, 16). The maximal developed force was recorded on a precalibrated Gould 2400 polygraph. To normalize force for differences in muscle size, muscle length at Lmax was measured at peak active force development, and cross-sectional area was determined from this length and the wet weight of the muscle and was expressed as g/mm^2. The results were compared with identical

measurements using papillary muscle from healthy, unburned animals (control group).

Myocardial edema—Full-thickness pieces of left and right ventricular wall were taken for measurement of tissue edema. The tissues were weighed, dried in an oven for 1 week, and then weighed again on consecutive days until the weight stabilized. Tissue edema is expressed as the ratio of tissue water weight to tissue dry weight.

Statistical analysis

Hemodynamic parameters and blood sample data were compared between groups at baseline using Student's *t* tests. A repeated measures ANOVA for incomplete data using an unstructured covariance matrix was used to analyze time-dependent changes within and between groups. Pretreatment cardiac work index was used as a covariate in the analysis of LCWI and RCWI. LAPs were used as covariates in the analysis of treatment effect on CI and LVSWI. Unless stated otherwise, *p* values in the text refer to Wald tests of significance of fixed effects and covariates (BMDP 5V, BMDP Statistical Software, Inc., Los Angeles, CA).

RESULTS

Initial effects of burn

Results are reported as mean ± SE. All animals entering the study lived for 24 h after burn and were included in the analyses. Sheep recovered from anesthesia and were extubated within 15–45 min after burn; all measurements were taken with the animals standing in their cages. An average 35% decrease in LCWI (paired *t* test, $p = .0001$) and RCWI (paired *t* test, $p = .0002$) developed during the first hour after burn in all animals (Fig. 1). The decreased work indexes were primarily related to a 42% fall in CI from baseline (paired *t* test, $p = .002$, Fig. 2). A decrease in filling pressures (LAP, CVP) to approximately one-half of baseline values (Table 1) and an increase in HCT to 37 ± 1.4% from a preburn value of 30 ± .8% were also evident 1 h after burn. On average, MAP increased 6 mmHg 1 h after burn, and HR increased from 93 to 129 (Table 1). Systemic and pulmonary vascular resistance indexes increased 90% and 112%, respectively, from preburn values. Oxygen delivery was decreased, O_2 consumption remained unchanged, and O_2 extraction increased 35% during the first hour after burn. (For a complete discussion of the above parameters, see Reference 18). The heart-specific plasma markers troponin I and CKMB were below detectable levels before burn in all animals (Table 2), whereas both of these compounds were elevated in plasma after burn. Although peak levels of each marker were higher in the NS group than in the HSD group, these differences were not significant over time or between groups. Plasma markers of general tissue damage (ALT, AST, and CK) and for tissue oxidative injury (plasma TBARS) were within the normal range at baseline and were markedly increased after burn. The levels of all these markers were similar in the two groups, suggesting that the degree of injury was comparable (Table 2). Plasma K^+ increased significantly in both groups after burn (Table 1).

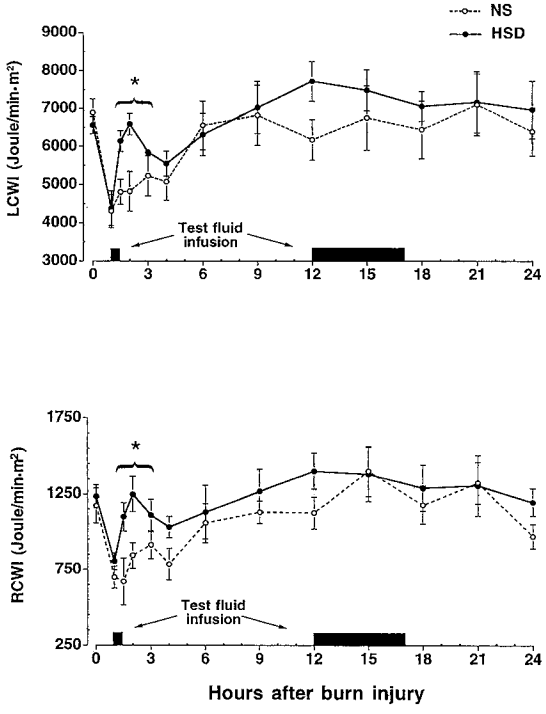

FIG. 1. **Left and right cardiac work indexes.** Data are displayed as mean ± SE. Infusion of HSD 1 h after burn restored LCWI and RCWI to preburn levels; the effect lasted 2–3 hours. Time · treatment interaction effects are significant for the time period 1.5 h through 3 h inclusive, marked by a bracket with an asterisk: $p = .04$ for LCWI; $p = .0001$ for RCWI.

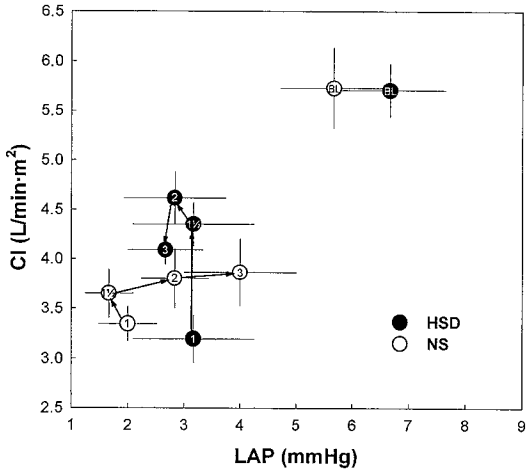

FIG. 2. **CI versus preload.** Data are displayed as mean ± SE. BL = baseline. Each circle represents CI plotted against LAP at a particular time point before (BL) or after burn (1, 1.5, 2, and 3 h). Burn produced a sharp drop in CI as well as in filling pressure. The development of the CI:LAP relationship is shown for each group for subsequent time points, connected with arrows. Test fluids were given between 1 h and 1.5 h after burn. CI in the HSD-treated group improved substantially without a corresponding increase in filling pressure, demonstrating a positive inotropic effect. The opposite is the case for the NS group, where filling pressures increased without a corresponding increase in CI. Time · treatment interaction effect, $p = .0003$; repeated measures ANOVA with LAP as covariate.

Treatment effects

Hemodynamic and cardiac effects—Start of resuscitation did not cause changes in blood pressures or HR in any of the treatment groups (Table 1). CI increased 45% from pretreatment values within 30 min after the end of the initial HSD dose and was restored to 80% of baseline, whereas it only increased 15% (to 67% of baseline) after NS (time · treatment interaction, $p = .005$, Table 1). The marked increase in CI during and after HSD infusion ($p = .0001$) occurred without a concomitant increase in filling pressure, whereas, on the other hand, the increase in filling pressure ($p = .027$) after NS was not

TABLE 1. **Hemodynamic variables and serum electrolyte concentrations**

	Group	Baseline (Preburn)	1 h (After Burn, Before Treatment)	2 h (After First Treatment)	6 h	12 h (Before Second Treatment)	24 h (After Second Treatment)
HR (beats per min)	HSD	89 ± 3.3	126 ± 14.7 *	120 ± 7.4 *	99 ± 8.7 *‡	84 ± 5.7	95 ± 7.7
	NS	96 ± 5.9	132 ± 6.2	133 ± 8.5	107 ± 7.2	94 ± 9.7 ‡	104 ± 10.9 ‡
MAP (mmHg)	HSD	92 ± 3.6	103 ± 5.3	109 ± 4.5	108 ± 5.8 *	108 ± 3.1 *	104 ± 6.6 *
	NS	95 ± 3.4	97 ± 7.4	96 ± 5.6 *	101 ± 4.8	101 ± 2.9	98 ± 5.2
PAP (mmHg)	HSD	22 ± 0.9	22 ± 1.0	23 ± 1.6	24 ± 2.1	24 ± 0.9	22 ± .6
	NS	21 ± .8	19 ± 1.2	21 ± 1.2	22 ± .9	23 ± 1.1	21 ± 1.1
LAP (mmHg)	HSD	7 ± 1.0	3 ± 1.1 *	3 ± .9 *	4 ± .5	5 ± 1.4	6 ± 1.2
	NS	6 ± 1.0	2 ± .5	3 ± .6	5 ± .9 ‡	6 ± 1.2 ‡	6 ± 1.5 ‡
CVP (mmHg)	HSD	6 ± 1.0	4 ± .9 *	3 ± .7 *	5 ± .9	5 ± 1.1	5 ± .8
	NS	6 ± .8	4 ± 1.2	4 ± .7	7 ± 1.5	5 ± 1.3	7 ± 1.8
CI (L/min · m^2)	HSD	5.7 ± .27	3.2 ± .24 *	4.6 ± .27	4.5 ± .25 *‡	5.5 ± .29	5.2 ± .37
	NS	5.7 ± .40	3.4 ± .17	3.8 ± .30 *§	5.1 ± .35	4.7 ± .35 ‡	5.1 ± .36 ‡
SVI (mL/stroke · m^2)	HSD	64 ± 3	27 ± 3 *	39 ± 3	47 ± 4	68 ± 7	56 ± 6
	NS	60 ± 4	25 ± 1	29 ± 3 *§	48 ± 4 *‡	53 ± 6 ‡	51 ± 5 *‡
Plasma Na^+ (mEq)	HSD	148 ± 1	149 ± 2	154 ± 1	150 ± 1	148 ± 0	144 ± 1
	NS	145 ± 1	145 ± 1	143 ± 2 §	145 ± 2	145 ± 1	143 ± 1
Plasma K^+ (mEq)	HSD	3.9 ± .10	4.4 ± .23 *	3.3 ± .16	3.4 ± .09 *‡	3.6 ± .07 *‡	3.9 ± .12
	NS	4.1 ± .19	4.8 ± .56	4.0 ± .29 ‡	3.3 ± .32	3.6 ± .15	4.0 ± .25 ‡
Plasma Cl^- (mEq)	HSD	115 ± 1	115 ± 1	123 ± 2	119 ± 1	120 ± 1	116 ± 1
	NS	114 ± 0	112 ± 1	112 ± 1 §	114 ± 2	116 ± 1	114 ± 1

Values are expressed as mean ± SE. Preburn samples were collected immediately before anesthesia and burn, and 1 h samples immediately before treatment. A repeated measures ANOVA was used: *$p < .05$ compared with baseline (effect of burn on both groups); ‡$p < .05$ compared with burn (effect of treatment on both groups); §$p < .05$ for time · treatment interaction compared with burn (effect of HSD vs. NS).

TABLE 2. **Plasma markers of tissue injury**

		Baseline	1 h	4 h	15 h	24 h
Troponin I (ng/g protein)	HSD	<5.6	10.2 ± 1.70*	14.5 ± 2.24*	24.0 ± 4.73*	20.9 ± 4.74*
	NS	<5.6	11.6 ± 1.02*	17.1 ± 3.46*	34.0 ± 17.55*	23.8 ± 9.43*
CK-MB (ng/g protein)	HSD	19.0 ± 10.31‡	33.7 ± 13.59	26.9 ± 4.54	64.6 ± 15.37	33.8 ± 10.47
	NS	<5.6	25.6 ± 13.60*	27.1 ± 10.62*	47.0 ± 29.44*	50.4 ± 38.05*
CK (total) (IU/g protein)	HSD	7 ± 1.8	204 ± 41.1*	641 ± 129.4*	856 ± 181.3*	896 ± 171.5*
	NS	4 ± 1.1	130 ± 26.7*	669 ± 168.6*	838 ± 156.1*	794 ± 151.9*
ALT (IU/g protein)	HSD	.14 ± .016	.38 ± .056*	.92 ± .203*	1.56 ± .360*	2.09 ± .410*
	NS	.14 ± .027	1.76 ± .120*	.76 ± .163*	1.44 ± .320*	1.73 ± .380*
AST (IU/g protein)	HSD	1.4 ± 0.19	3.7 ± .63*	10.7 ± 2.45*	26.4 ± 6.14*	31.5 ± 5.71*
	NS	1.6 ± .17	3.7 ± .58*	11.8 ± 3.45*	22.5 ± 5.88*	25.1 ± 6.85*
TBARS (nmol MDA/g protein)	HSD	27.9 ± 3.4	96.5 ± 9.9*	95.4 ± 13.3*	88.9 ± 8.8*	58.7 ± 7.0*
	NS	31.5 ± 2.6	90.1 ± 6.8*	85.8 ± 10.9*	71.6 ± 9.5*	65.5 ± 8.6*

Values are expressed as mean ± SE. *$p < .05$ (t test) with respect to baseline. ‡The value is influenced by one outlier at this time point. The amounts of troponin I and the cardiac-specific enzyme CK-MB in plasma after burn were increased from baseline values, but were not influenced by HSD treatment. Plasma levels of CK, ALT, and AST reflect general cell injury after burn; TBARS levels reflect tissue oxidative stress. Values are expressed in units per g plasma protein to correct for the changes in plasma volume caused by burn and treatment and for the plasma-expanding effect of HSD.

associated with a statistically significant increase in CI (Fig. 2). An analysis of the changes in CI with LAP as covariate confirmed that the increase in CI after HSD could not be explained by augmented preload, suggesting an HSD-induced increase in cardiac contractility (time · treatment interaction, $p = .0003$ for the time points shown in Fig. 2). The use of CI divided by LAP as an index of contractility was considered acceptable, because neither preload nor afterload changed over the examined time period. This apparent effect of HSD on contractility was limited to the first 2 h after treatment start. Thereafter, both CI and LAP gradually increased in all animals regardless of treatment regimen (Table 1) and returned to preburn values by 15 h in both groups. In the HSD-treated group, HCT decreased 7% to 30 ± 1% ($p = .022$, t test), thus restoring it to baseline values. Meanwhile, HCT decreased only 2% to 36 ± 1% (not significant) in the NS group, indicating continued hemoconcentration in this group.

Cardiac work—Left and right cardiac work indexes were restored to preburn values during HSD infusion, whereas in the NS group, work indexes did not improve (Fig. 1). The treatment effect persisted for 2 (LCWI) to 3 (RCWI) hours after the end of infusion (time · treatment interaction, $p = .004$ for LCWI; $p = .035$ for RCWI) and then receded. Cardiac work was subsequently gradually restored to preburn values or above in both groups by 6 h after burn, likely due to the continued fluid resuscitation with LR. SVI also increased significantly after HSD infusion (time · treatment interaction, $p = .01$, Table 1). The second, slow HSD infusion at 12 h after burn did not further augment cardiac performance; at 24 h after burn, CI, SWI, and SVI were similar in both groups and had returned to near baseline values.

Cardiac contractility index—We designed an index to estimate contractility *in vivo* by taking the ratios of stroke work to preload (LVSWI:LAP and RVSWI:CVP) and comparing

changes in these ratios to changes in the filling pressures alone. Filling pressures and stroke work indexes were both reduced 1 h after burn; however, the initial HSD infusion rapidly improved stroke work indexes in both left and right ventricles despite sustained low filling pressures (Fig. 3). An analysis of the LVSWI:LAP ratio using LAP as a covariate clarified the direct inotropic effect of HSD on left cardiac work capacity, independent of Starling mechanisms. A time · treatment · LAP interaction (p = .038) persisted from end of treatment through 4 h after burn in the HSD group, but not the NS group (Fig. 3). Corresponding changes were seen in the right ventricle, as the RVSWI:CVP ratio increased markedly after treatment in the HSD group, but not the NS group. An analysis of the RVSWI:CVP ratio using CVP as covariate yielded a time · treatment · CVP interaction (p = .022) over the same time span (Fig. 3).

MBF—Preburn MBF was distributed between the different regions of the heart as expected, with the greatest perfusion in left ventricular endocardium; there were no significant differences between groups with regard to MBF (Fig. 4). Myocardial perfusion was significantly diminished 60 min after the first treatment dose (2 h after burn) in all three regions of the heart in the NS group, whereas MBF was maintained at baseline levels in the HSD group (Fig. 4). All subsequent measurements of myocardial perfusion were similar to preburn values in both groups regardless of treatment intervention.

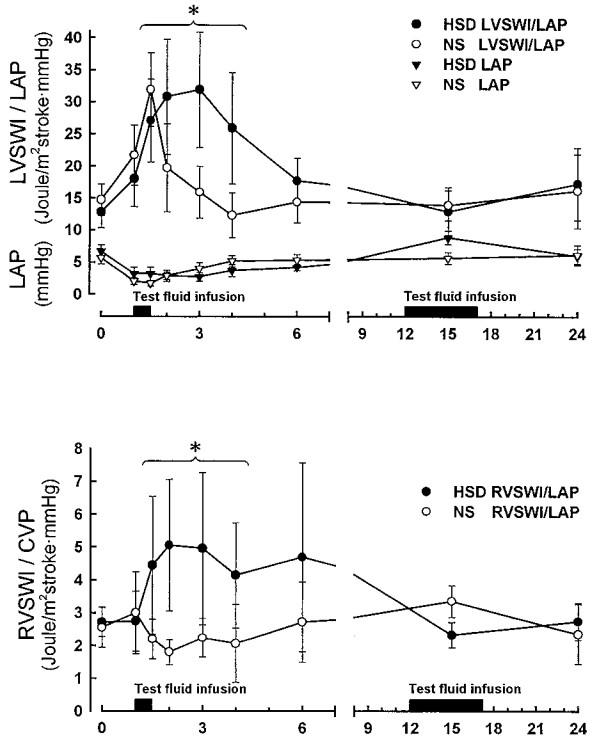

FIG. 3. **An *in vivo* contractility index based on the relationships LVSWI/LAP and RVSWI/CVP shows persistent increase in ventricular contractility for more than 4 h after HSD treatment.** Data are displayed as mean ± SE. For the left ventricle, in addition to being incorporated into the index, LAP values are displayed separately in the bottom tracing, to better appreciate their contribution to the index values. Contractility was greater in the HSD group, although filling pressures were not different between groups; time · treatment · LAP (p = .038) and time · treatment · CVP (p = .022) interaction effects using filling pressures as covariates are significant for the time period 1.5 h through 4 h inclusive, marked with brackets and asterisks.

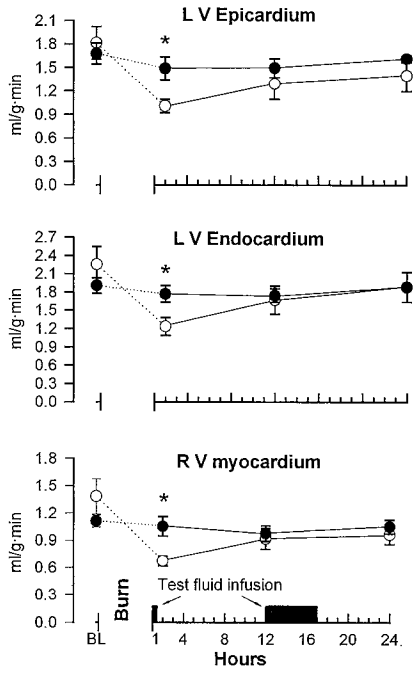

FIG. 4. **MBF.** Data are displayed as mean ± SE. BL = baseline, LV = left ventricle, RV = right ventricle. MBF was maintained at preburn levels throughout the recovery period after HSD treatment, while it was greatly diminished 1 h after the first treatment dose in the NS group. *p (t tests) for differences (Δ BL −1 h) between groups: LV epicardium, p = .06; LV endocardium, p = .04; RV, p = .004.

Myocardial maximal contraction force—Right papillary muscles were harvested at the end of the experiment from all sheep for *ex vivo* study. Muscle cross-sectional area was 1.78 ± .35, 1.82 ± .20, and 2.23 ± .48 mm^2 (mean ± SE) for control, HSD, and NS groups, respectively. Although some muscles had a cross-sectional area greater than 2 mm^2, one study reported (17) that mechanical responses of thicker papillary muscles to hypoxia were the same as those of thinner muscles, suggesting that an anaerobic core may be present in some thicker muscles, yet its presence may not affect the mechanic responses. In our experiments, we maintained partial pressure of oxygen above 500 mmHg, and the peak developed tension remained constant for several hours. Contracture developed in a few muscles during the stabilization period, indicating hypoxia, and those specimens were discarded. Maximal contraction force developed by papillary muscle was significantly lower in the NS-treated burned group compared with controls consisting of healthy unburned animals (Fig. 5). In contrast, maximum contraction force measured in papillary muscle from HSD-treated burned sheep was not different from that measured in the unburned control group (t test, p = .11) and was significantly higher than that measured in the NS-treated burned group (t test, p = .015).

Plasma markers of tissue injury—Troponin I and CKMB remained elevated throughout the treatment and recovery period, and there were no consistent or treatment-specific patterns for the elevated enzyme levels. Troponin I levels in burned sheep (average < 2 ng/mL) were lower than the levels described in humans with a similar degree of burn (usually >

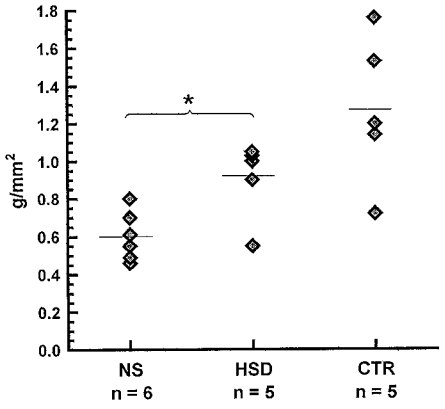

FIG. 5. **Maximum contraction force in right ventricle papillary muscle.** NS = normal saline-treated group, HSD = hypertonic saline dextran-treated group, CTR = nonburned control group. The maximum contraction force in right ventricular papillary muscle from each animal is displayed as a diamond. Horizontal lines represent the group means. Despite considerable overlap in the data, it is likely that the means represent an underestimate of the *in vivo* contraction force, as specimens producing the lowest contraction forces may have been damaged by environmental factors during tissue sampling and testing. The maximum contraction force was greater in HSD-treated animals than in NS-treated animals 24 h after burn. *$p = .016$ (t test).

2 ng/mL) (6). Plasma CK, ALT, AST, and TBARS also remained elevated or were increasing throughout the treatment and recovery period, without time- or treatment related differences between groups for any of these compounds (Table 2).

TBARS and antioxidant status of myocardium—TBARS levels in left ventricle 24 h after burn were 20% higher compared with those of tissue from unburned controls (Table 3). This change was accompanied by a similar 20% decrease in the peroxidation potential after burn injury, but the difference was only significant in the NS group compared with control (Table 3). In addition, glutathione concentrations in left ventricle were about 50% lower in burned sheep treated with NS than in unburned control animals. Although there was no significant difference in TBARS levels or lipid peroxidation potential in left ventricle from burned sheep treated with HSD when compared with NS-treated sheep, the glutathione concentrations in HSD-treated animals were significantly higher and closer to control values. In right ventricle, TBARS levels were unaffected by burn injury, whereas glutathione concentrations were significantly lower than controls in both burn groups (Table 3). Some significant differences appeared in the activities of the antioxidant enzymes GPx, GR, SOD, or catalase in left or right ventricle for either HSD- or NS-treated animals when compared with unburned controls (Table 4). In the cases where antioxidant enzyme activities were significantly different between the HSD and NS groups, the activities in the HSD group were similar to controls (Table 4).

Miscellaneous—Darkened, in many instances opaque black, urine occurred during the first 1–5 h after burn, but gradually resolved. There was no difference in onset or duration of this phenomenon between groups, and all urine collections were clear in all animals within 12–16 h after burn. Plasma sodium increased on average 5 mEq/L after HSD and did not increase above 157 mEq/L in any animal at any time (Table 1). Base excess, pH, and blood gases remained within normal limits for the duration of the experiment (data not shown). The ratio of cardiac muscle water to dry tissue weight at the end of the study was not different between the burned groups and not different to unburned animals (data not shown). A burn-induced increase in fluid requirement occurred in most animals, which presented itself several hours earlier in the NS group than in the HSD group, as determined by our urine output-driven infusion regimen (18). However, the total fluid volume infused at 24 h was similar in both groups (100 mL/kg in NS vs. 92 mL/kg in HSD). After the initial treatment with test solution, the burn-induced plasma K^+ values returned to baseline levels in the NS group but decreased to levels significantly below baseline levels in the HSD group ($p = .016$, t test). After 2 more hours, both groups were hypokalemic with respect to preburn levels, but K^+ gradually returned to baseline by 24 h (Table 1). Plasma Cl^- levels were proportionate to plasma Na^+, except after HSD treatment, when Cl^- increased relatively more than Na^+ ($p = .02$, t test).

DISCUSSION

The present study evaluated the effect of giving two infusions of HSD in addition to standard isotonic resuscitation on cardiac function after a large thermal injury. The effects of burn were evident from the decrease in filling pressures and the increase in HCT. Cardiovascular dysfunction was evident from the decrease in CI, cardiac work indexes, and the *in vivo* contractility index. The observed improvements in cardiac parameters after the initial HSD treatment dose support the hypothesis that HSD offers cardioprotection after burn injury. We had postulated that the second, slowly infused dose of HSD 12 h after burn would enhance the cardioprotective effects of the first dose. Although data collected 12–24 h after burn trauma did not directly support this assumption, an overall beneficial effect of HSD on the heart was nevertheless maintained by the greater maximal contraction force generated by papillary muscle isolated from HSD-treated animals 24 h after burn.

TABLE 3. **Myocardial tissue markers of cardiac oxidative injury**

	Left Ventricle			Right Ventricle		
	HSD (n = 6)	NS (n = 5)	Control (n = 4)	HSD (n = 6)	NS (n = 4)	Control (n = 5)
TBARS (nmol MDA/mg protein)	4.6 ± .32*	4.2 ± .21	3.5 ± .38	1.7 ± .25	1.6 ± .24	1.5 ± .22
Glutathione (nmol/mg protein)	40.1 ± 2.82*‡	25.7 ± 1.04*	55.5 ± 4.89	57.1 ± 2.25*	61.6 ± 4.62*	72.6 ± 3.23
Peroxidation potential	3.0 ± .31	3.1 ± .21*	3.8 ± .17	–	–	–

Effect of burn and resuscitation on heart glutathione and TBARS levels in sheep. The control group consists of myocardial tissue from five unburned animals. Values are expressed as mean ± SE. *$p < .05$ (t test) with respect to control group. ‡$p < .002$ (t test) with respect to NS group. Peroxidation potential is calculated as the ratio of Fe^{2+}-stimulated TBARS levels to basal levels and reflects the ability of the tissue to undergo further lipid peroxidation.

TABLE 4. **Myocardial antioxidant enzyme activities**

	Left Ventricle			Right Ventricle		
	HSD (n = 6)	NS (n = 5)	Control (n = 4)	HSD (n = 6)	NS (n = 4)	Control (n = 5)
GPx	.94 ± .072	.78 ± .053	.82 ± .031	.31 ± .050‡	.47 ± .023*	.36 ± .035
GR	.16 ± .016*	.14 ± .011	.12 ± .005	.04 ± .006‡	.06 ± .004	.05 ± .003
Mn SOD	30.3 ± 3.12	29.9 ± 3.38	29.4 ± 3.00	13.0 ± 0.87‡	17.6 ± 2.53*	12.6 ± 1.14
Cu Zn SOD	32.9 ± 2.75‡	23.3 ± 2.41	28.9 ± 2.73	19.3 ± 1.94	24.5 ± 2.55	23.3 ± 2.07
Catalase	194 ± 16*	158 ± 14	147 ± 3	126 ± 7	132 ± 24	99 ± 9

Effect on burn and resuscitation on heart antioxidant enzyme activity in sheep. Data expressed in U/mg protein as mean ± SE. *$p < .05$ (*t* test) with respect to control group. ‡$p < .05$ (*t* test) with respect to NS group.

Our findings are in agreement with studies by Horton et al. (19), who found that a single dose of HSD, infused either immediately, 1 h or 4 h, but not 8 h after burn trauma, improved cardiac contractility as measured 24 h after burn in a Langendorff preparation. In the clinical setting, early patient access is often not possible; therefore, it is important to explore how long after burn trauma HSD treatment can be given and still offer benefit. In the present study, the second dose was begun 12 h after burn based on our assumption that the "time window" for HSD-mediated cardioprotection might be extended throughout the postburn period, by having given the initial dose early. Dextran 70 has been shown to have a half-life of 7 h in rabbits and about 10 h in pigs, whereas plasma Na^+ returns to preinfusion levels about 3 h after HSD infusion (9). We estimated that the volume-expanding effect of HSD would be over by 12 h and, therefore, the second dose was given at this time.

Myocardial depression with left or right ventricular failure is a serious complication in burns, particularly in older patients with less cardiac reserve, and in the young burn patient with an immature sympathoadrenal axis (20). Recent studies have shown the importance of appropriate cardiac monitoring during treatment of burn patients regardless of age (20, 21). However, some limitations to the measurement of ventricular function in the shock subject, including shock-related changes in preload, afterload, and HR must be considered when interpreting our results (22). Although cardiac work indexes may yield useful information as to whether the maximum work capacity is diminished after burn, the changes are not readily evident from the hemodynamic measurements routinely available in clinical burn units. Collecting hemodynamic data in the resting state may fail to unmask burn-related cardiac dysfunction as well as HSD-related effects on the heart. However, despite these limitations in assessing cardiac contractility in human burned subjects, our finding of improved cardiac work indexes and MBF suggests an important benefit of early HSD treatment of burn injury. At this time, CI, cardiac work, and MBF were higher and were closer to preburn values in the HSD group. CI, cardiac work, and MBF were similar in both groups from 6 to 24 h after burn. This suggests that although cardiac reserves were less in the NS group, this difference did not translate to reduced global indexes of hemodynamic function with the animal in the resting state.

Previous studies have described a negative inotropic effect of hypertonic NaCl (300–1800 mOsm) when injected directly into the coronaries of healthy mongrel dogs (23). Similarly, Brown and colleagues reported that increasing osmolarity with NaCl alone in isolated hearts produced a negative inotropic effect (5). However, in the same study, improved post-ischemia-reperfusion cardiac contractile performance was found when 7.5% NaCl was given in combination with 6% dextran, and the investigators attributed the beneficial effect of HSD treatment to the ability of the dextran component to scavenge free radicals in the heart (5). Our finding of a positive inotropic effect of HSD as indicated by the LVSWI:LAP and RVSWI:CVP contractility indexes in burned sheep is consistent with studies reported by Suzuki et al. (4), who used a model of 50% TBSA scald burn injury in anesthetized dogs and reported that an initial dose of HSD (4 mL/kg) injected intravenously provided transient hemodynamic changes, whereas a second dose given 5.5 h later provided no added benefit. However, Suzuki and colleagues showed that HSD resuscitation increased preload and reduced afterload without significant changes in cardiac contractility as measured by end-systolic pressure-volume relationship and preload-recruitable stroke work (4). To account for the absence of similar changes in our study, it is important to note the differences in the delivery regimens that were used: 4 mL/kg injected over 20 s in the experiment of Suzuki et al. as opposed to the same volume infused over 30 min in the present study. This makes for a difference in electrolyte influx that is 90 times higher in the Suzuki et al. study, producing a very large, very transient stepwise change in plasma sodium concentration and osmolarity, as opposed to a gradual, long-lasting, small change. The reduced afterload and augmented preload reported by Suzuki et al. occurred so soon after HSD infusion, and was of short duration (< 20 min), that similar changes would hardly be noticed in the present study, where the first measurement took place at the end of the 30 min HSD infusion. Other factors, like differences in time to treatment (30 vs. 60 min), species (dog vs. sheep), and state of consciousness (anesthetized vs. conscious) may also have contributed to the observed differences.

Our finding of improved contraction force in papillary muscle harvested 24 h after burn and HSD treatment is in agreement with improved cardiac contractility after HSD, as reported by Horton and colleagues (3). Although results were obtained with papillary muscles from the right ventricle only, it is likely that comparable changes were present in the left ventricle, as the cardiac work indexes and contractility indexes were similarly affected on both sides. The role of sympathetic activation and catecholamine release in HSD treatment of burns is not well defined. In humans, burn injury increases circulating norepinephrine but not epinephrine (24). Hypertonic saline (NaCl 8%) treatment has been shown to increase

circulating norepinephrine severalfold in conscious rats subjected to 60 min hemorrhagic hypotension (25), whereas HSD infusion had the opposite effect in conscious sheep subjected to the same level of hemorrhagic hypotension for 180 min (thus having reached higher levels of circulating norepinephrine before treatment) (26). An HSD-related increase in circulating catecholamines could either increase the force of myocardial contraction directly or improve cardiac performance secondary to an increase in MBF, consistent with our current findings. Catecholamine levels were not determined directly in the present study.

Plasma levels of the myocardium-specific regulatory protein troponin I have been shown to increase progressively for 24 h after major burn injury in rabbits (27) and in humans (6). Troponin I can be detected in the blood 3–4 h after myocardial damage and may persist in the circulation for 5–7 days. Murphy et al. (6) detected levels in excess of 2 ng/mL measured 4 h after burn in patients resuscitated with LR only, whereas patients receiving a single 4 mL/kg dose of HSD within 6 h of injury had significantly lower plasma troponin I levels. Although plasma troponin I levels rose after burn trauma in the present study, there were no statistically significant differences between treatment groups for either troponin I, CK, or CKMB.

At present the mechanisms responsible for the myocardial depression seen after burn remain unknown. Previous studies have suggested the existence of a myocardial depressant factor associated with burn injury, but a specific agent has not been identified (19, 28). In the present study, we investigated whether myocardial depression after burn was related to free radical generation and lipid peroxidation of myocardial membranes. It has been observed that TBARS and conjugated diene levels increase in plasma after burn injury (7, 8, 29) and that treatment with antioxidants may be beneficial (29–31). The data from the present study suggest that increased lipid peroxidation occurs in response to burn trauma. In heart tissue, some changes in antioxidant enzyme activities, glutathione concentrations, and peroxidation potential were observed in burned animals compared with controls. These data suggest that free radical mechanisms may be involved in the cardiac depression observed after burn. In addition, the present study suggests that there are differences in the levels of antioxidants expressed per mg protein between the left and right ventricles. To our knowledge, this aspect of cardiac metabolism has not received much attention and warrants further study with respect to the response of the heart to trauma and injury.

The increase in plasma K^+ observed in most animals immediately after burn may be due to burn-induced hemolysis. The rapid decrease in plasma K^+ subsequent to HSD has been reported in earlier studies (25) and may in part be due to circulating norepinephrine, but largely the reason for this induced hypokalemia remains unexplained. The reason for the observed increase in plasma Cl^- relative to Na^+ after HSD treatment is not known.

It is questionable whether the two-dose HSD regimen described in this experiment offered an advantage over a single initial infusion. The maximum benefit may occur with early initial HSD infusion only, and the lack of "carry-over" benefit from the first to the second dose in our experiment may be due either to the time delay between the doses or to the much slower infusion rate (4 mL/kg over 5 h vs. 4 mL/kg over 30 min) for the second HSD dose, or both. Although multiple doses of HSD may prove to be beneficial in large burns, it would seem more appropriate to give the second dose earlier, for example, soon after the effect of the first dose on cardiac variables subsides (around 3 h), but before the time shown by others to have limited beneficial effects (Suzuki et al. (4) ,6 h for anesthetized dogs, Horton et al. (19), 8 h for burned guinea pigs). In addition, sustained cardioprotection using a second HSD dose will likely require a more rapid infusion rate, similar to that used for the initial bolus. Dividing the total amount of HSD into two separate 30 min infusions may result in a slightly hyperosmotic state in the plasma, without the risk of developing dangerous levels of hypernatremia or hypervolemia.

It seems justified to believe that giving a second dose of HSD earlier would make a difference in the cardiac response to the treatment. In the natural development of burn shock, pathophysiological changes take place most rapidly in the beginning. Capillary leak is most prominent shortly after burn, and lymph flow increase from the burned area displays a biphasic response (32). Edema is maximal around 6 h after noninhalation burn injury and only starts to resolve after 12–23 h (33). We believe there is a greater chance of influencing these changes with hypertonic solutions while they actually develop, than later, when their effect in the tissues has become manifest.

In summary, this blinded, prospective trial showed beneficial effects of an initial dose of HSD on cardiac performance the first 24 h after a large burn injury. A second, slow infusion given after 12 h was without measurable effect on hemodynamic variables, yet isolated papillary muscles studied 24 h after burn trauma confirmed the cardioprotective effects of HSD. Although cardiac depression may contribute to the overall morbidity and mortality in burns, particularly in older patients or in the very young, the clinical importance of HSD in providing cardioprotection remains to be determined. Further trials evaluating different hypertonic infusion regimens as well as efficacy and timing of a subsequent dose are justified.

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