Use of Nuclear Magnetic Resonance Spectroscopy to Assess Renal Dysfunction After Hypertonic-Hyperoncotic Resuscitation in Rats

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Background: The aim of this study was to evaluate the renal tolerance of a hypertonic-hyperoncotic solution (HHS) administration during uncontrolled hemorrhagic shock (UHS).

Methods: UHS was produced in rats by a preliminary bleed followed by tail amputation. Hydroxyethylstarch (HHS) 200/0.5 6% in NaCl 7.2% was administered to the HHS groups (n = 20) and normal saline (NS) to the NS group (n = 20). Infusion rates were adjusted to prevent mean arterial pressure (MAP) from falling either below 40 mm Hg in the HHS40 (n = 10) and NS40 groups (n = 10), or below 80 mm Hg in the HHS80 (n = 10) and NS80 groups (n = 10). Data obtained were compared with a sham group and a no resuscitation (NR) group. Nephrotoxicity was evaluated by nuclear magnetic resonance analysis in urine samples.

Results: Survival was 60% in the NS40 group and 40% in the NS80 group, 70% in the HHS40 group, and 60% in the HHS80 group (p = not significant). Within and between target groups of 40 mm Hg MAP and 80 mm Hg MAP, there was no significant difference in survival. The mean values of renal metabolites to creatinine (ct) ratios were not significantly different among the six groups. Principal component analysis showed that the HHS80 group was characterized by an increase in allantoin/ct and urea/ct ratios demonstrating acute renal dysfunction and failure of nitrogen metabolism.

Conclusion: In prolonged UHS, an infusion of HHS may not increase the rate of survival. HHS infusion in normotensive resuscitation appears to be associated with renal toxicity.

Key Words: Fluid therapy, Shock, Hemorrhage, Nephrotoxicity, Magnetic resonance spectroscopy.


Fluid resuscitation is the principal initial treatment of uncontrolled hemorrhagic shock (UHS). The Advanced Trauma Life Support course, based on American College of Surgeons guidelines, suggests fluid resuscitation and early definitive surgical treatment. 

Despite these recommendations, the choice of fluid, the time to start the fluid management, and the blood pressure goal during initial fluid challenge are open to debate. Several animal studies have shown that a fluid resuscitation with a low blood pressure goal decreases rebleeding and mortality. Massive fluid expansion used to increase blood pressure toward normal levels may lead to worse outcome through disruption of early thrombus formation, coagulopathy, hypomodulation, and rebleeding. Conversely, hypotensive resuscitation with a small fluid challenge seems to reduce the risk of death in all animal trials in which it has been investigated.

A hypertonic-hyperoncotic solution (HHS) offers an alternative approach in the early treatment of UHS. Its osmotic properties draw fluid into the intravascular compartment, where the addition of colloid (dextran or hydroxyethylstarch) helps to prolong its effects through binding of the recruited water. This approach has been used in initial resuscitation of hemorrhage in a few studies. A recent meta-analysis of 14 human randomized trials showed a tendency toward decreased mortality without statistical significance where a hypertonic solution was used. Reported mortality in animal experiments using HHS is contradictory and appears to depend on the model of UHS. In particular, a fixed dose bolus infusion of hypertonic saline in UHS has been reported to increase arterial pressure and blood loss to unpredictable levels and to decrease survival in rats and pigs. A recent report showed no survival benefit in UHS in rats by HHS treatment. Clearly, further studies are necessary to elicit any potential benefits over standard fluid therapy.

Nevertheless, HHS is of significant interest, because of its effects on hemodynamic and inflammatory responses. Hypertonic saline treatment substantially elevates mean arterial pressure (MAP) and cardiac output with a marked increase in renal, mesenteric, and splanchnic perfusion.
Furthermore, renal hypoperfusion, which occurs in hemorrhagic shock, creates an environment in which cellular injury and organ dysfunction can be produced during the episode of shock. We aimed to determine renal tolerance after 180 minutes of hemorrhagic shock and to evaluate the effects of restoration of blood volume with normal saline (NS) versus HHS on survival.

Thus, this study examined both nephrotoxicity and the effect on survival outcomes of treatment of UHS with HHS compared with NS in two blood pressure goal strategies: normal blood pressure and hypotensive resuscitation. We hypothesized that the infusion of HHS during fluid resuscitation would improve survival time and survival rate with no signs of nephrotoxicity in these two blood pressure strategies.

**MATERIALS AND METHODS**

**Animal Preparation**

Male Wistar rats were exposed to a UHS outcome model previously described. Briefly, this model subjects the animals to UHS in two phases (UHS phase I of 90 minutes, resuscitation phase II of 90 minutes). Before the experiment, the rats were housed in groups of three in standard cages and kept with food and water available ad libitum in a temperature-controlled room (22°C) on a 12-hour dark and light cycle. All experiments were performed in accordance with the French laws regarding animal experimentation.

After anesthesia with pentobarbital (35 mg/kg intraperitoneally) and ketamine (60 mg/kg intramuscularly), using a neck incision, polyethylene catheters were introduced into the carotid artery for hemodynamic monitoring and the jugular vein for resuscitation. Through a paw incision, a polyethylene catheter was introduced into the femoral artery for exsanguination and blood sampling. The animals were kept supine during the experiments, and body temperature was monitored with a rectal thermistor and maintained by a thermostatically controlled heating pad at 38.0°C ± 0.5°C. The carotid line, containing a calibrated pressure transducer, was directly connected to a controlled data acquisition system (Biopac MP30, BIOPAC Systems Inc., Goleta, CA). Heart rate was computed from the arterial tracing. Total preparation time was approximately 60 minutes.

Arterial blood samples of 0.25 mL were drawn for monitoring pH, Pao₂, PaCO₂, standard bicarbonate, base excess, oxygen saturation, hematocrit, hemoglobin, sodium, potassium, lactate, and blood urea nitrogen at baseline.

In phase I, UHS was initiated by pump-controlled withdrawal of arterial blood, 3 mL/100 g during 15 minutes. The shed blood was preserved in a sterile condition in an anticoagulant acid-citrate-dextrose solution for later re-infusion. At 20 minutes, uncontrolled hemorrhage was produced by tail amputation of 75% of its length. The bleeding tail was immediately directed into a graduated tube.

At 30 minutes postinitiation of UHS, the rats were randomly assigned into one of six groups of 10 rats: the sham group (anesthesia, cannulation of the animal’s carotid and femoral arteries and jugular vein), the NR group (no resuscitation), the HHS40 group (titrated infusion of HHS to prevent decrease in MAP below 40 mm Hg), the NS40 group (titrated infusion of NS to prevent decrease in MAP below 40 mm Hg), the HHS80 group (titrated infusion of HHS to prevent decrease in MAP below 80 mm Hg), and the NS80 group (titrated infusion of NS to prevent decrease in MAP below 80 mm Hg). The HHS solution (Rescueflow, Biophau-sia Inc., Stockholm, Sweden) consisted of hydroxethyl starch (HES) 200/0.5 6%, dissolved in NaCl 7.2%. This solution has an osmolarity of 2,464 mosm/L⁻¹.

In phase II, hemostasis was achieved by tail wound cautery and closure. Simultaneously, fluid resuscitation was undertaken with blood and NS solution to achieve MAP ≥70 mm Hg and a hematocrit ≥30% during 90 minutes. Cannulation sites were then closed. At the end of this phase, surviving rats were killed by lethal pentobarbital infusion and urine samples were withdrawn.

**Magnetic Resonance Spectroscopy**

**Samples**

Samples of 1 mL urine were collected from all rats included in the study and kept at −20°C until magnetic resonance spectroscopy (MRS) analysis. Samples were obtained at the end of the experimentation immediately after the rat’s death.

**MRS Analysis**

On the day of MRS analysis, samples were thawed at room temperature. A volume of 0.6 mL of urine was placed in a 5-mm diameter MRS tube together with 0.1 mL of D₂O containing a known amount of fumaric acid. The fumaric acid resonance at 6.53 ppm in urine proton spectra was used as a reference for the chemical shifts. Proton spectra acquisition was performed with a 500-MHz spectrometer (Varian Inc, Palo Alto, CA) at 25°C and equipped with a sample changer. The proton spectra were obtained with 64 transients of 90-degree pulses and 2.5-second relaxation delay. The water signal was suppressed by a presaturation pulse of 0.03 mW applied during the relaxation delay. The spectral width was 6 KHz on 16,000 data points. Fourier transformation was applied without zero filling with an exponential window function corresponding to 0.1 Hz line broadening. The region between 0.5 ppm and 8.5 ppm was analyzed. The resonance areas of formic acid, hippuric acid, urea, allantoin, taurine, trimethylamine-N-oxide (TMAO), creatinine (ct), dimethylglycine, dimethylamine, citrate, succinate, N-acetyl-neuraminidase, N-acetylglutamime, acetate, alanine, and lactate were measured respectively at 8.4 ppm, 7.6 (and 3.98) ppm, 5.8 ppm, 5.4 ppm, 3.26 ppm, 3.24 ppm, 3.05 (and 3.90) ppm, 2.96 ppm, 2.72 ppm, 2.70 ppm, 2.39 ppm, 2.06 ppm, 2.04 ppm, 1.92 ppm, 1.48 ppm, and 1.33 (and 4.13 ppm). The signal areas were determined from processed spectra and compared with the area of the ct resonance at 3.05 ppm.
ppm. When no resonance could be measured for a metabolite, a zero value was recorded for this compound.

**Statistical Analysis**

Data were compared using the Student’s *t* test or Mann-Whitney *U* test, where appropriate. Survival rates were compared using Fisher’s exact test. To detect a difference of 60% in survival (probability 0.8 vs. 0.2, power 0.8), a sample size estimation called for a minimum of 10 animals per group. Data were expressed as mean ± SEM, unless otherwise indicated. A *p* value <0.05 was considered statistically significant.

**MRS Statistical Analysis**

A multivariate data analysis by principal component analysis (PCA) was used for comparing the 60 urine samples from the six rat groups. The PCA procedure was used to discriminate metabolic profiles for all urine samples. The multidimensional space obtained was described by two or three axes. Better discrimination could be achieved with axis 1 and axis 2.

The statistical analyses were performed using SAS software version 6.12 (SAS Institute Inc., Cary, NC).

**RESULTS**

**Baseline Characteristics**

All six groups were comparable regarding baseline characteristics, with no statistically significant differences found in weight, physiologic, or laboratory parameters (Table 1).

**MAP Evolution**

Evolution of MAP for all groups is represented in Figure 1. After the initial blood withdrawal at up to 15 minutes postinitiation of UHS, MAP continued to decrease in all groups reaching 27 ± 12 mm Hg in the HHS40 group, 25 ± 6 mm Hg in the NS40 group, 23 ± 4 mm Hg in the NS80 group, 26 ± 12 in the HHS80 group, and 23 ± 4 mm Hg in the NR group. Up until 20 minutes, at the time of the tail cut, the MAP of all studied groups did not differ statistically. We observed no difference between the HHS80 and NS80 groups regarding the evolution of MAP or any statistical difference between the HHS40 and NS40 groups. Concerning the NR group, MAP decreased gradually with time. The MAP of groups included in the normotensive fluid challenge groups (HHS80, NS80) had a significantly higher MAP than did rats included in the hypotensive resuscitation groups during phase II of the experiment (*p* < 0.0001).

**Blood Loss**

Total blood loss shed from the tail wound is represented in Figure 2 for each group. Total blood loss did not differ significantly in group HHS80 versus group NS80 or in group HHS40 versus group NS40. On the other hand, there was a significant difference between groups HHS80 and NS80 (61 ± 60 mL/kg) compared with groups NS40 and HHS40 (11 ± 10 mL/kg, *p* < 0.001).

**Fluid Volume Requirement**

Total fluid volume required was significantly larger in groups treated with isotonic saline than in groups infused with HHS in the two targets. The titrated fluid volume required to reach MAP 40 mm Hg was 6 ± 3 mL/kg with HHS versus 59.1 ± 31.2 mL/kg with NS (*p* < 0.001). Regarding the 80 mm Hg target, the HHS group required 14.4 ± 6.2 mL/kg versus 157.9 ± 58.9 mL/kg in the NS group (*p* < 0.001).

**Survival**

All rats included in the sham group survived. In the NR group, all rats died quickly, before 90 minutes. There was no significant group difference in survival rate at 180 minutes for the other four groups. With respect to target groups of 40 mm Hg MAP (groups HHS40, NS40) and 80 mm Hg MAP (groups HHS80, NS80), respectively, there were also no differences between groups (Fig. 3). Survival at 90 minutes

**Table 1** Baseline Measurements in the 6 Groups of Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>NR</th>
<th>HHS40</th>
<th>NS40</th>
<th>HHS80</th>
<th>NS80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>357 ± 37</td>
<td>346 ± 15</td>
<td>355 ± 7</td>
<td>356 ± 14</td>
<td>368 ± 14</td>
<td>348 ± 12</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>428 ± 17</td>
<td>415 ± 14.3</td>
<td>432.7 ± 12.4</td>
<td>433.5 ± 16.8</td>
<td>398.7 ± 21.2</td>
<td>398.7 ± 21.2</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>133 ± 7</td>
<td>120 ± 4</td>
<td>125 ± 4</td>
<td>129 ± 5</td>
<td>123 ± 5</td>
<td>121 ± 6</td>
</tr>
<tr>
<td>Arterial blood</td>
<td>7.41 ± 0.03</td>
<td>7.42 ± 0.03</td>
<td>7.38 ± 0.01</td>
<td>7.40 ± 0.01</td>
<td>7.36 ± 0.01</td>
<td>7.39 ± 0.06</td>
</tr>
<tr>
<td>pH</td>
<td>43 ± 1</td>
<td>42 ± 3</td>
<td>47 ± 2</td>
<td>37 ± 2</td>
<td>47 ± 2</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>75 ± 0.4</td>
<td>74 ± 2</td>
<td>79 ± 5</td>
<td>74 ± 2</td>
<td>73 ± 0.5</td>
<td>80 ± 0.4</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>2.5 ± 3.5</td>
<td>3.5 ± 2.5</td>
<td>3.2 ± 2.2</td>
<td>−0.7 ± 2.0</td>
<td>−0.3 ± 0.3</td>
<td>−0.75 ± 2.2</td>
</tr>
<tr>
<td>BE (mmol/L)</td>
<td>3.8 ± 0.1</td>
<td>3.4 ± 0.3</td>
<td>3.6 ± 0.2</td>
<td>3.6 ± 0.2</td>
<td>3.6 ± 0.2</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>136.5 ± 0.5</td>
<td>142.3 ± 0.8</td>
<td>142.5 ± 2.3</td>
<td>140.6 ± 2.2</td>
<td>139.5 ± 3.0</td>
<td>140.7 ± 2.5</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>0.93 ± 0.16</td>
<td>0.71 ± 0.18</td>
<td>0.86 ± 0.11</td>
<td>0.89 ± 0.06</td>
<td>0.68 ± 0.12</td>
<td>0.68 ± 0.12</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.25 ± 0.05</td>
<td>0.33 ± 0.03</td>
<td>0.3 ± 0.1</td>
<td>0.25 ± 0.02</td>
<td>0.26 ± 0.03</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39.0 ± 1.0</td>
<td>40.4 ± 1.7</td>
<td>37.0 ± 3.2</td>
<td>37.0 ± 1.7</td>
<td>34.7 ± 1.4</td>
<td>38 ± 1.1</td>
</tr>
</tbody>
</table>

Results are displayed as mean ± SEM.

BE, base excess; MAP, mean arterial pressure.
was 6 of 10 rats (60%) in group NS40 and 4 of 10 rats (40%) in group NS80, 7 of 10 rats (70%) in group HHS40, and 4 of 10 rats (40%) in group HHS80 (p = not significant). Concerning the time of survival, all fluid-treated groups (groups HHS and NS) showed significantly better survival times than did the untreated group (group NR) with 46.1 ± 18.4 minutes versus 19.7 ± 10 minutes (p < 0.001). Groups with MAP targets of 40 mm Hg (HHS40 and NS40) showed better survival time than did those with an MAP target of 80 mm Hg (HHS80 and NS80) with 53 ± 13 minutes versus 38 ± 20 minutes (p < 0.05). There were no significant differences between groups with the same blood pressure targets, i.e. between groups NS40 and HHS40, with 48 ± 15 minutes versus 57 ± 5 minutes, respectively, or between groups NS80 and HHS80, with 40 ± 21 minutes versus 37 ± 21 minutes.

**Physiologic Variables at the End of Hemorrhagic Shock**

Physiologic variables at the end of UHS (90 minutes) did not differ between groups with the exception of hematocrit, serum sodium, and lactate. Lactate level was significantly lower in group NS80 compared with the HHS80 group (3 ± 2.6 mmol/L vs. 6 ± 2.9 mmol/L, p < 0.03). Serum sodium was significantly higher in the HHS80 group (172 ± 12 mm/L) compared with in the NS80 and other groups. At the end of phase I, hematocrit was higher in the NS40 group (20.7% ± 6.4%) than in the NS80 group (11.3% ± 3.1%).

**Renal Tolerance**

**Dosage of Urine Metabolites**

An example of nuclear magnetic resonance (NMR) spectra is represented in Figure 4 with the assignments of the main metabolite peaks. The mean values of metabolite to ct ratios were not significantly different among the six groups. The values obtained for urea, allantoin, lactate, and taurine are represented in Figure 5.

**Principal Component Analysis**

In contrast, the principal component analysis (PCA) allowed discrimination of four different regions (Fig. 6). Axis 1 (abscissa) is representative of kidney status being mainly composed of allantoin (70%) and urea (15%), but dependent on liver metabolism, with taurine (5.8%) and anaerobic metabolism (lactate 3.3%). Axis 2 (ordinate) is also representative of kidney status being composed of urea (71%), allantoin (15%), and of liver metabolism through the contribution of hippurate (4%); the contribution of citrate (3%) is representative of both organs.

This PCA can discriminate four regions with these two axes describing 74% of the variance. Region 1 includes 13 urine samples, among them 8 from rats of the sham group, corresponding to 61% of urine samples of this region. Consequently, this region is representative of the control values. Region 2 includes 83% of samples from rats without treatment, and those treated with HHS40 or with NS40. Thus, this
region is representative of hypotensive fluid resuscitation. Region 3 corresponds to 57% of urine samples from rats resuscitated with NS with an 80 mm Hg blood pressure target. Region 4 corresponds to 89% of urine samples from rats resuscitated with HHS with an 80 mm Hg blood pressure target. Discrimination of region 2 from region 1 is characterized by a decrease of urea/ct ratio, corresponding to the lack of nephrotoxicity. Discrimination of region 4 from region 1 is characterized by an increase of allantoin/ct and urea/ct ratios, demonstrating acute renal dysfunction and failure of nitrogen metabolism.

**DISCUSSION**

In our study, we failed to demonstrate a significant benefit with regard to survival by use of HHS versus NS in the resuscitation of UHS using either a hypotensive or a normotensive strategy. As for renal tolerance, our results indicate that the use of HHS is associated with renal toxicity when used in larger doses (HHS80 group).

Recent studies have challenged current guidelines for the initial management of hemorrhagic shock. Initial hemorrhagic shock treatment, where bleeding cannot be controlled, is the subject of debate regarding optimal blood pressure targets and the type and quantity of fluid resuscitation. Massive intravenous fluid administration causes hemodilution, as well as an increase in the extracellular fluid compartment; it may cause disruption of hemostatic clots in damaged blood vessels, thereby causing further bleeding. Results have varied with the type of model but limited fluid resuscitation has been shown to be of benefit in multiple animal studies. In contrast, aggressive resuscitation with large volumes of isotonic saline or lactated Ringer’s solution increased bleeding and mortality.

Anesthetic agents may have an impact on with outcome in the model used in this study. Nevertheless, the response of rats under pentobarbital anesthesia more closely approximates that of unanesthetized rats than other anesthetics such as the combination of droperidol and ketamine. Moreover, an animal hemorrhage model should take into consideration the interaction between anesthesia and the tested treatment because most severe trauma patients receive anesthetic agents early in their care.

Although we observed no statistical difference in the rate of survival among any of the treated groups, there is a significant increase in time of survival in the rats managed with hypotensive resuscitation compared with in normotensive rats. The power of our study did not permit us to detect a significant mortality difference of less than 60%. The lack of a significant difference concerning the mortality may be explained by the small sample size used. Thus, the benefit of the hypotensive strategy remains unclear. Dutto et al. randomized 110 hypotensive patients with suspected continuing hemorrhage to a target systolic blood pressure of more than either 100 mm Hg or 70 mm Hg. They found no difference in survival. In contrast, a meta-analysis including 44 animal studies found a beneficial effect using hypotensive pressure strategies. Another recent animal study found that the systolic blood pressure required for provoking new bleeding was 90 mm Hg, independent of the start of bleeding.

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*Fig. 4. Proton NMR spectra obtained on a rat urine sample with assignment of main metabolites (spectral region from 1 to 4.5 ppm).*
Several animal models of uncontrolled bleeding have found that an increase in systolic blood pressure increases bleeding.\textsuperscript{3,18,21} Our study is in accordance with these results because the two groups with a target of 80 mm Hg (HHS80 and NS80) suffered significantly greater blood loss than did groups managed with hypotensive resuscitation (Fig. 2). Our

**Fig. 5.** Mean value determined by magnetic resonance spectroscopy in urine of urea/ct (A), allantoin/ct (B), lactate/ct (C), and taurine/ct (D). There are no significant differences between groups.

**Fig. 6.** Principal component analysis map of 4 regions. Region 1, urines of low pressure rats (*○,●*); region 2, sham; region 3, NS80; and region 4, HHS80. (∆) sham; (*) NR; (∘) NS40; (●) HHS40; (□) NS80; (■) HHS80.
Renal Dysfunction After Resuscitation

data agree with those published by Kentner et al., who did not demonstrate enhanced blood loss in UHS treated with HHS compared with lactated Ringer’s solution.14 Interestingly, fluid requirements were very different between the normo-
tensive resuscitation groups, with HHS80 requiring 14.4 ±
6.2 mL/kg−1 and NS80, 157.9 mL/kg−1 (p < 0.001). This
fact suggests that the level of blood pressure should be con-
sidered only as an indicator of blood loss and not of the
amount of volume required.

Various modes of fluid resuscitation have been evaluated
using different models of UHS. The use of HHS was pro-
posed by Velasco et al. as the sole nonblood resuscitation
fluid for the treatment of hemorrhagic shock in patients.22,23
Addition of colloid to hypertonic saline helps to prolong its
effects through the bonding of the recruted water.24 In our
present study of UHS in rats, we demonstrated that hypoten-
sive and normotensive resuscitation of UHS can be per-
formed with the infusion of HHS. We failed to demonstrate a
benefit in survival with HHS versus NS, however. Kentner
et al., using the same model, found no difference in survival in
“permissive hypotensive” resuscitation between HHS and
lactated Ringer’s solution.14 Our study demonstrates that it
was possible to maintain a normotensive blood pressure with
a “small volume resuscitation” strategy. This fact is important
because the maintenance of a high value of blood pressure is
fundamental for patients with hemorrhage and brain injury to
optimize cerebral perfusion pressure near 70 mm Hg. Al-
though the effect of HHS on survival of patients with severe
brain injury is unclear, re-analysis of individual data from
previous studies found an improvement in survival of hypo-
tensive patients with head injury, where it acts to increase
cerebral perfusion pressure.25 A recent study found a superi-
ority of HHS solution compared with 20% mannitol in pa-
ients with an intracranial pressure higher than 20 mm Hg.26
Other beneficial effects include osmotic force to bring water
from the interstitial space of the brain parenchyma into the
vascular compartment, improvement of cerebral flow and
oxygen delivery, decreasing endothelial cell edema.27 We
chose to titrate the infusion of HHS because Kentner et al.
recently demonstrated, in a model of UHS resuscitated with
permissive hypotension, that titrated intravenous infusion of
HHS can maintain controlled hypotension without increasing
blood loss.14

Lactate levels after resuscitation were higher in the group
receiving HHS to achieve a MAP of 80 mm Hg than in other
groups. This fact was probably the result of the impairment of
hepatic function as reflected by increase of taurine/ct and
lactate/ct in urine samples in NMR analysis.

In our study, we found similar results if we compared the
fall of hematocrit level in groups treated with HHS with those
treated with isonotic saline. Mechanisms of hemodilution
were presumably different between HHS and NS. Hemodil-
ution with HHS is thought to be caused by movement of
interstitial fluid into vessels. The mechanism for isonotic saline
was simply a volume expansion by exogenous fluid.

Resultant hemodilution was similar and oxygen flux would
be expected to have fallen with both fluids.

Finally, early treatment of UHS must have as a principal
objective the avoidance of cardiac arrest secondary to major
hypovolemic, but also to rapidly minimize shock status and
the decrease in oxygen transport. Profound UHS in our study
resulted in significant mortality. In this context, it was pos-
sible that fluid resuscitation alone was inadequate.

Nephrotoxicity

The use of MRS has been widely used to investigate the
renal function in patients and in animals.28-31 The advantage
of this technique was the possibility to understand the mecha-
nism of the nephron impairment in relation to the metabolite
detected by MRS in the urine sample in contrast to ct mea-
surement. The association of nephrotoxicity with HES use
was noted when renal transplant recipients who received
kidneys from brain dead donors given HES developed acute
renal failure (ARF).32 Controversy persists surrounding this
association with some reports refuting any connection of HES
with renal failure.33 Recently, several studies provide data to
support the concept that HES therapy is associated with the
development of renal dysfunction.34 A multicenter study evalu-
ated the effects of HES and gelatin on renal function in
patients with severe sepsis.35 ARF, oliguria, and peak serum
tconcentrations were significantly higher in the HES group
than in the gelatin group. Multivariate analysis revealed that
HES administration was a risk factor for ARF. HES seems to
cause renal dysfunction through the development of proximal
tubular swelling and vacuolization (osmotic nephrosis). HES
is taken up by proximal tubular cells, where it is unable to
undergo degradation by the cell. Intracellular HES then
generates an oncotic gradient across the cell membrane that
causes intracellular accumulation of water and cellular
swelling.34 Low molecular weight HES with a low degree of
substitution (HES 200/0.5) and doses under 33 mL/kg−1/day−1
seem to improve renal tolerance.34

In our study we administered HHS containing hydroxy-
ethylsarch 200/0.5 in both high (14.4 ± 0.6 mL/kg−1 in the
HHS80 group) and low volumes (6.2 ± 0.3 mL/kg−1 in the
HHS40 group). We chose to use NMR spectroscopy to eval-
uate nephrotoxicity, as it has been shown to be an efficient
tool to evaluate renal status in a number of clinical settings.30
Proton NMR spectroscopy associated with a multivariate
analysis is an appropriate method for detection of metabolite
such as allantoin, taurin, and ct in the urine of rats.36 In renal
transplantation, this method was able to discriminate between
transplant rejection and cyclosporine nephrotoxicity.29 This
method, associated with multiparametric statistical analysis,
was also able to localize the anatomic region of antibiotic
nephrotoxicity.30,37 In the present study, the NMR parameters
were analyzed using PCA.38,39 Our results demonstrated,
through multivariate analysis, the discrimination of four dis-
tinct regions and mean values of urine metabolites did not
show any significant differences (Fig. 5).
The map obtained by use of PCA (Fig. 6) showed the importance of several parameters on the metabolic status of rats. In hypotensive resuscitation groups, we were unable to distinguish the group treated by HHS from the group treated by NS (region 2 of the PCA map, Fig. 6). In contrast, among rats included in the normotensive fluid resuscitation groups, PCA analysis discriminates two regions for HHS and NS. The HHS80 group (region 4) seems to be influenced by increased allantoin and urea elimination, whereas rats included in the NS80 group have a metabolic profile closer to reference samples (region 1) than region 4. Overall, hepatic metabolism of HHS-treated rats seems to be impaired as taurine/ct and lactate/ct were increased and hippurate/ct was decreased (component of axis 1). These results may be compared with the recent study performed by Kentner et al. who monitored liver hypoxia in a rat model of UHS. They measured liver dysxia by analysis of liver surface PCO₂. They found an initial increase in liver PCO₂ with either HHS or LR infusion with no significant difference between the two groups. Unfortunately, they did not study fluid therapy maintaining normal blood pressures.

Urea and allantoin are the most useful metabolites to distinguish the urine samples. Urea is a metabolite of elimination of amino nitrogen common to mammalian species and the allantoin is the last metabolite in the ureogenesis cycle of nitrogen elimination in the rat. The samples from rats with normal blood pressure resuscitation and included in regions 1, 3, and 4, are characterized by higher urea/ct ratio values than are samples from rats with hypotensive resuscitation. This can be explained by two mechanisms. Significant production of nitrogenated metabolites is possible when liver can synthesize urea starting from the amino acids or by greater elimination of urea in urine through increased urinary flow related to increased renal blood pressure. However, variations related to urinary flow were eliminated by reporting the urinary amount of metabolites to urinary amount of ct for each urine sample. Creatinine is directly related to the glomerular filtration and thus, indirectly, to the blood flow. Therefore, the variation of urea/ct ratio is mainly related to the variation of nitrogen elimination.

Rats resuscitated with HHS using a normotensive strategy (HHS80 group) were characterized by an increased allantoin/ct ratio value. In rats, unlike humans, allantoin is the terminal purine catabolite, formed via oxidation of uric acid by uricase. Allantoin can be formed in the liver, lung, spleen, or kidney. Several studies of the renal function by analysis with NMR spectroscopy have shown that elimination of allantoin is in relation to renal function impairment. With lanthanum, a toxic rare earth, the amount of allantoin in urine is decreased in relation to dilution of urine because of a medullary loop deficiency. The medullary region deficiency was confirmed in this study by the increase of osmotic-aminated molecules (TMAO). In our study this region of the kidney seems to be preserved, as TMAO does not interfere with the PCA analysis. Consequently, the allantoin concentration and the urea concentration are not lowered by a deficiency in water reabsorption; on the contrary, they are increased. The effect of the perfusion we observed in our experimental model is rather likely related to the effects observed after ischemia or reperfusion of rat kidney grafts. Allantoin has been described as a marker of oxidative stress subsequent to ischemic or reperfusion injury in the rat kidney. It may be argued that allantoin is not a completely specific marker of renal damage as it is synthesized in the liver, but hepatic disturbances seem to decrease the levels of this metabolite. In our study, the concomitant increases of allantoin/ct and urea/ct ratios are in favor of our description of an impairment of the kidney rather than of hepatic insufficiency. The renal damage detected with the NMR analysis cannot be correlated with varying degrees of shock and resuscitation because of the difference in survival time because the NS80 and HHS80 groups presented similar survival times and renal impairment was detected only in the HHS80 group. Thus, infusion of a high volume of HHS is associated with renal damage likely via an ischemic reperfusion mechanism. In contrast, rats resuscitated with the same fluid, using a hypotensive strategy, did not reveal an increase of allantoin/ct values nor urea/ct values. Infusion of a small volume of HHS is apparently associated with acceptable renal tolerance.

CONCLUSION

We conclude that in prolonged UHS, a titrated infusion of HHS can maintain controlled hypotension or normotension with a significant reduction in infusion volume required, compared with that of NS. Unfortunately, this strategy may not increase the rate of survival, but the use of HHS is associated with longer duration of survival. Resuscitation with HHS with a normotension target seems to be associated with renal dysfunction explained by an ischemic or reperfusion process. Nevertheless, this renal damage was detected only by allantoin/ct ratio. Further studies are warranted to characterize the specific renal toxicity produced by requirements for a large volume of HHS during resuscitation of hemorrhagic shock.

REFERENCES

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