Effects of hetastarch and mannitol on prolonging survival in stable hypothermia in rats

TZE-FUN LEE, JEFFREY WESTLY, AND LAWRENCE C. H. WANG
Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

Lee, Tze-Fun, Jeffrey Westly, and Lawrence C. H. Wang. Effects of hetastarch and mannitol on prolonging survival in stable hypothermia in rats. Am J Physiol Regulatory Integrative Comp Physiol 278: R1040–R1047, 2000.—In rats, prolonged stable hypothermia (~24 h at body temperature of 19°C) is characterized by a time-dependent increase in hematocrit, plasma osmolality, and red blood cell fragility and a decrease in plasma volume. These changes impede tissue microcirculation and could limit survival. As a countermeasure, we used plasma volume expanders of both long (hetastarch) and short-lasting (mannitol) characteristics to improve microcirculation and hopefully hypothermia survival. Infusion of 6% hetastarch at hour 3 in hypothermia significantly (P < 0.05) enhanced survival over saline control (33.5 vs. 23.8 h); a significant delay in the increases of hematocrit and cell fragility was also observed compared with those in saline controls. Treating the animal with 6% hetastarch at hour 20 during hypothermia caused a similar but less-effective improvement in survival. In contrast, treating the rats with 6% mannitol at hour 3 or 20 during hypothermia failed to enhance survival over saline control, although transient improvement in plasma volume was observed. Our results indicate that by using a long-lasting volume expander, which tends to better maintain plasma volume and rheological parameters governing microcirculation than does saline or a short-lasting volume expander, hypothermia survival can be significantly improved.

microcirculation; volume expander; hematocrit; osmolality; plasma volume

WHEN EXPOSED TO SEVERE COLD, mammals increase their heat production to counter heat loss to maintain a constant body temperature (Tb). If heat loss surpasses maximum heat production, hypothermia results and, unless aided by external heat sources, death ensues. To develop proper treatments that would enhance hypothermia survival, it is of importance to understand what physiological changes may lead to death during hypothermia. Although the failure of respiratory and/or cardiovascular functions, renal functions, acid-base regulation, and ion regulatory mechanisms have been suggested as critical (4, 15, 23), there is no consensus as to what physiological deterioration actually limits survival during prolonged severe hypothermia.

A major problem in many previous studies on hypothermia survival is the instability of the hypothermic animal preparation, which often only lasts a few hours, rendering the evaluation of limiting factors for survival difficult to establish (14). Because of this inherent difficulty, we have developed an animal model in which stable hypothermia could be established for prolonged periods (24–120 h) (16). This model thus offers the investigation of various time-related deterioration in functions during prolonged hypothermia. Our previous findings indicated that a steady decrease in both the turnover and oxidation of glucose (14) and a time-dependent decrease in venous P02 in conjunction with an increase in plasma lactate were consistently observed during the progression of prolonged hypothermia (19). Taken together, these observations suggest that a gradual failure in circulatory insufficiency appears to be the limiting factor for survival. Supporting this contention are the marked declines in arterial blood pressure, heart rate, and cardiac output that would result in a marked depression of tissue blood flow during hypothermia (30). To further complicate the situation, changes in rheological characteristics of the blood can additionally impede tissue perfusion. For example, increases in whole blood viscosity (21, 24) and aggregation of erythrocytes and platelets (3) in hypothermia will further exacerbate an already compromised microcirculation.

Whereas it may be difficult to enhance hypothermia survival by overcoming the suppressive effect of low Tb on cardiovascular parameters, it may be possible to institute remedial measures to minimize the deleterious changes in rheological characteristics of the blood. This seems to be the right approach as we have previously shown that survival in hypothermia can be significantly prolonged by reducing platelet aggregation with EGTA (18). In the present study, we attempted to improve microcirculation with plasma volume expanders of long (hetastarch)- and short-lasting (mannitol) characteristics, which, by effects of hemodilution, may counteract increased cell aggregation and thus prolong survival in hypothermia.

METHODS

All experimental procedures have received prior approval of the University of Alberta Animal Use Committee and followed the guidelines of the Canadian Council on Animal Care. Three- to six-month-old male Sprague-Dawley rats, housed individually at an ambient temperature (Ta) of 22 ± 1°C under a 12:12-h light-dark photoperiod, were fed lab chow rationed to maintain the body weight near 400 g and given water ad libitum. All rats were maintained under this condition for 1–2 wk before starting the experiment. As the
RESULTS

The average survival time of the control rats treated with saline intravenously at either hour 3 or 20 after hypothermia was $\sim 23-27$ h, about the same as we reported earlier (16, 19). As shown in Table 1, treating the animals with 6% hetastarch 3 h after hypothermia significantly (P < 0.05) increased survival time to 33.5 h. Interestingly, treating the rats with the same concentration of hetastarch at hour 20 after hypothermia also increased survival time from 26.7 to 32.5 h; however, this difference did not achieve statistical significance (P = 0.1; Table 1). In contrast, infusion of mannitol (6%), which is isosmotic to hetastarch, at either 3 or 20 h after hypothermia did not elicit any beneficial effects in prolonging survival time. In fact, treating the rats with mannitol at the later stage of hypothermia (i.e., 20 h after hypothermia) appeared to have a serious deleterious effect as two of six rats receiving the infusion died within 15-30 min thereafter.

Table 1. Effects of intravenous infusion of 5 ml of saline, 6% hetastarch, or 6% mannitol (infused at either hour 3 or 20 after Tb of rat had reached 19°C) on duration of survival in prolonged hypothermia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of Survival in Hypothermia, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused at hour 3 after hypothermia</td>
<td></td>
</tr>
<tr>
<td>Saline (n = 8)</td>
<td>23.8 ± 1.21</td>
</tr>
<tr>
<td>Hetastarch (n = 8)</td>
<td>33.5 ± 2.25*</td>
</tr>
<tr>
<td>Saline (n = 6)</td>
<td>23.6 ± 1.94</td>
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<tr>
<td>Mannitol (n = 6)</td>
<td>27.7 ± 2.38</td>
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<tr>
<td>Infused at hour 20 after hypothermia</td>
<td></td>
</tr>
<tr>
<td>Saline (n = 8)</td>
<td>26.7 ± 2.25</td>
</tr>
<tr>
<td>Hetastarch (n = 8)</td>
<td>32.5 ± 2.69</td>
</tr>
<tr>
<td>Saline (n = 6)</td>
<td>25.2 ± 1.77</td>
</tr>
<tr>
<td>Mannitol (n = 6)</td>
<td>24.1 ± 1.46</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from concurrent saline control (P < 0.05). n, Number of rats; Tb, body temperature.
control rats, hematocrit did not change significantly until hour 5 in hypothermia and then increased significantly with time throughout the remainder of the hypothermic bout (Fig. 2A). Immediately after infusing hetastarch, hematocrit at hour 5 decreased significantly (P < 0.05) compared with the control value. The hematocrit value of the hetastarch-treated rats, although showing a progressive increase with time, remained significantly lower than that of the control rats at hour 20. The plasma osmolality also increased steadily throughout the hypothermic bout in the control rats (Fig. 3), similar to that observed with hematocrit. Treating the animal with hetastarch curtailed the rise in osmolality, resulting in a rightward shift of the curve (Fig. 3), which was marginally different from the controls (x coefficients: 1.42 ± 0.16 and 1.03 ± 0.14 for control and hetastarch, respectively, P = 0.09, n = 8). The plasma volume of the control rats decreased drastically (~30%) 3 h after hypothermia and then decreased continuously with time throughout the hypothermia bout (Fig. 2C). Treating the animal with hetastarch significantly (P < 0.05) reversed the decline in plasma volume at hour 5 (Fig. 2C). Although it did not achieve any significant difference, the plasma volume of the hetastarch-treated group remained higher than the control values at hour 20 (Fig. 2C). The fragility of the
red blood cells also increased with time in the control rats (Fig. 2D). Treating the rats with hetastarch significantly (P < 0.05) reduced the fragility of the red blood cells at hour 20 (Fig. 2D). At expiration, all rheological parameters were about the same in all treatment groups (Fig. 2).

The effects of infusing hetastarch at hour 20 after hypothermia on the rheological changes were summarized in Fig. 4. The patterns of rheological changes after infusing hetastarch at hour 20 were similar to those observed after infusing at hour 3; that is, slight reductions against the rising trend of hematocrit and cell fragility as well as a slight reversion of the decrease in plasma volume observed in the control rats. However, none of these changes achieved statistical significance.

Figure 5 shows the changes in hematocrit, osmolality, plasma volume, and cell fragility in rats treated with 6% mannitol at hour 3 after hypothermia. Within 2 h, the decrease in plasma volume was significantly reversed (P < 0.05; Fig. 5C); however, the values of both the control and the mannitol-treated rats were about the same at hour 20 (Fig. 5C). Similar to that observed in rats treated with hetastarch at the same time (hour 3), mannitol reduced the increases in hematocrit and osmolality immediately after perfusion (i.e., at hour 5). However, the increases in hematocrit and osmolality were about the same as the control rats at hour 20 after hypothermia (Figs. 5A and 3). Treating the animal with mannitol at hour 3 after hypothermia did not cause any change in cell fragility (Fig. 5D).

Figure 6 shows the rheological changes in rats treated with mannitol at hour 20 after hypothermia. In contrast to those observed with mannitol given at hour 3, infusion of mannitol at hour 20 did not cause any change in all rheological parameters examined.

**DISCUSSION**

Although the survival time of the control rats receiving intravenous saline at either hour 3 or 20 after hypothermia varied from 23 to 27 h (Table 1), there was no significant difference among the four control groups, indicating that infusion of saline at different hypothermic stages did not affect the duration of survival. This suggestion is further supported from our previous findings that the survival time (~24 h) of rats receiving no treatment (16, 19) is about the same as those observed in the present study. Collectively, these results indicate the stability and reproducibility of the animal model used. Our observed time-dependent increases in hematocrit and osmolality and a decrease in plasma volume strongly suggest that deleterious changes in the rheological characteristics of the blood were limiting tissue microcirculation and probably hypothermia survival. One of the most striking observations in the present study was the drastic (30%) decrease in plasma volume within the first 3 h of hypothermia (Figs. 2C, 4C, 5C and 6C). This decrease is unlikely due to the effect of low temperature on the method of our plasma volume measurement, because we have validated the technique against temperature effect alone (Fig. 1). A similar decrease in plasma volume has also been reported in rats (30), hamsters (28), and dogs (25) 2–6 h after the animal had become hypothermic. Classically, cold-induced hypovolemia is explained by an increased diuresis due to inhibition of antidiuretic hormone release (20). However, recent studies have added the shift of water from vascular to interstitial space due to increased blood pressure (cold-induced peripheral vasoconstriction) as another factor in the reduction of plasma volume in early hypothermia (7, 31).
Associated with the decrease in plasma volume, progressive increases in hematocrit and osmolality were also observed in hypothermic rats. It has been reported that a 3% increase in hematocrit without changes in temperature can result in a 10% increase in blood viscosity and a decrease of Tb from 37°C to 25°C can further increase the blood viscosity by another 38% (6). Thus a steady increase in hematocrit during hypothermia will lead to a continuing and substantial increase in blood viscosity. When coupled with low blood volume and cardiac output (30), the increasing viscosity is likely to further impede microcirculation in hypothermia. In addition, the fragility of the red blood cells was observed to increase with time in hypothermia (Fig. 2D). A decrease in cell deformability, aided by cell debris from damaged cells, is expected to further exacerbate an already deteriorated microcirculation in hypothermia. It is thus reasonable to suspect that this progressive deterioration in tissue perfusion will lead to poor tissue oxygenation, cellular substrate delivery, and utilization (14, 19), leading to expiration of the animal.

As a countermeasure, it is hypothesized that the hypothermic animal should survive longer if the dele-
rious changes in microcirculation can be minimized. One of the simplest treatments in improving microcirculation is to dilute the whole blood by increasing plasma volume, which will decrease hematocrit and lower blood viscosity. However, infusion of fluid does not always reverse cold-induced hemococoncentration. It has been shown previously that saline infusion had minimal lasting effects and did not enhance cardiovascular recovery from hypothermia (11, 25). This may be due to the rapid clearance of saline from the intravascular space. Conversely, infusing the hypothermic dog with a 10% low molecular weight dextran increased plasma volume and decreased the sludging of blood (11). However, the duration of survival was not addressed in this study. Furthermore, dextran may not be a suitable option for enhancing hypothermia survival because of its relatively short-lasting effect (2–4 h) (26) and its antigenicity due to its bacterial origin (22). In the present study, we used hetastarch, another volume expander, but with much longer duration of action (24 h) (26) and excellent clinical safety record (25) to enhance hypothermia survival. Treating the animal with hetastarch at the early stage of a hypothermic bout (3 h after hypothermia) significantly prolonged the survival time by almost 10 h (41%). The major difference between the hetastarch- vs. saline-treated rats is the reduction of the observed increase in hematocrit at hour 5 and beyond (Fig. 2A). Because of the long-lasting effect of hetastarch, the hematocrit remained significantly lower than the control value even after 20 h in hypothermia (Fig. 2A). The importance of maintaining a near-normal hematocrit to survive hypothermia is demonstrated in our previous study that in the Richardson’s ground squirrels, in which hematocrit does not change during most of a hypothermia bout, can survive at a Tb of 7°C approximately three times as long as can the rat at a Tb of 19°C (19). In addition to delaying the rise of hematocrit, hetastarch also reduced the increases in cell fragility (Fig. 2D). As hetastarch has been shown to improve erythrocyte deformability and prevent erythrocyte aggregation both in vitro and in vivo (9, 12, 29), it may have some direct effect in minimizing cell fragility. Furthermore, hetastarch may also retard the increases in hematocrit and cell fragility by better maintaining the plasma volume than saline.

It is of great interest to note that when survival time is correlated with plasma volume taken at hour 20, having received various treatments at hour 3, a positive correlation (r² = 0.61, P < 0.001, n = 28) was observed (Fig. 7A). In fact, all animals survived >30 h typically associated with a plasma volume >20 ml/kg, and animals that received hetastarch survived the longest. A similar conclusion can also be drawn by comparing the survival time with plasma volume obtained at hour 22 having received various treatments at hour 20 (Fig. 7B, r² = 0.82, P < 0.001, n = 28). Again, a plasma volume >20 ml/kg appears to segregate the long survivors versus the short survivors, and those receiving hetastarch survived the longest (Fig. 7B). Thus regardless of the timing of the hetastarch treatment, the critical consideration appears to be whether a critical plasma volume (~20 ml/kg) can be maintained to ensure better survival. Indeed, the few individuals that received either saline or mannitol and were able to maintain a high plasma volume, also survived longer (Fig. 7, A and B).

Although it is difficult to quantify the proportional importance of hetastarch on enhancing rheological characteristics versus plasma volume per se, the longer survival in hypothermia attested to the functional benefit of this treatment. The patterns of rheological changes after treating the animal with hetastarch at hour 20 were similar to those observed at hour 3, but to a lesser extent (Fig. 4). Consequently, the survival time increased by ~6 h. The inability to achieve statistical significance for this treatment could be due to the longer survival time of the control group (26.7 vs. 23.8 h for the hour 3 control group). However, it is also possible that 32–33 h is the optimal survival time that can be achieved by the concentration of hetastarch used in the present study. Collectively, our results demonstrated that treating the animal with hetastarch can prolong hypothermia survival by delaying the progressive deterioration of microcirculation.
Mannitol (6%) has also been shown to increase plasma volume after administration (1, 33). When given at the early stage of hypothermia, similar effects on rheological changes as those observed with hetastarch were seen (Fig. 5). However, in contrast to those observed with hetastarch, the changes in hematocrit, plasma volume, and osmolality after mannitol were short-lived and by hour 20, became indistinguishable as those seen in the controls. Consequently, the mannitol-treated rats survived about the same length of time as the saline controls. The failure of mannitol to maintain a long-lasting effect could be due to its relative short elimination half-life (<2 h) (2, 8). Thus a critical consideration in using plasma expanders to enhance hypothermia survival is the duration of its action; a longer-acting one is significantly more beneficial than a shorter-acting one. Unexpectedly, infusing mannitol (6%) at the later stage of hypothermia appeared to have a serious deleterious effect as two of six animals died shortly after infusion. Although not certain why this should be so, the osmotic effect of mannitol, which can cause water to be drawn from erythrocyte (8, 32) and further decrease cell deformability, could exacerbate an already impeded microcirculation. In addition, mannitol-induced circulatory overload due to expansion of the vascular volume has been reported to cause pulmonary edema and congestive heart failure in patients with diminished cardiac reserve (10, 32). As the cardiac function is markedly reduced in the later stage of hypothermia, these combined adverse effects could further impair survival of the animal.

In conclusion, during hypothermia, a high hematocrit and increased blood viscosity, coupled with a concomitant decrease in cardiac output and an intense peripheral vasoconstriction, are conducive for the aggregation of blood cells in capillary beds leading to poor tissue perfusion. In particular, hemoconcentration may reduce cerebral blood flow and compound the depressant effects of low Tb on central regulation of cardiovascular and respiratory functions. Because only hetastarch, but not mannitol, prolongs survival at low Tb, it is of importance to employ a long-lasting volume expander to maintain the patency of microcirculation in hypothermia. Because of the excellent clinical safety of hetastarch, our observed results may encourage further studies in improving microcirculation and prolonging long-term clinical hypothermia survival.

Perspectives

Although numerous perturbations occur during profound and prolonged hypothermia, the critical factors limiting long-term survival in hypothermia remain unclear. On the basis of the observations from the present and our previous studies, we are of the opinion that the maintenance of proper microcirculation is the key factor in determining the survivorship under prolonged hypothermia. With the proper remedial measure such as using a long-lasting plasma expander (hetastarch) to maintain patency of microcirculation in hypothermia, we have demonstrated significant improvement in hypothermia survival. Enhancing long-term survival in the whole animal also indicates a successful maintenance of organ and tissue function under profound hypothermia. With this in mind, our present findings could provide useful applications not only on the recovery of victims from accidental hypothermia, but also in the long-term preservation of organs to allow time for best donor/recipient tissue matching before transplantation.

The present study was supported by a grant from Natural Sciences and Engineering Research Council of Canada to L. Wang (A-6455).

Address for reprint requests and other correspondence: L.-C. H. Wang, Dept. of Biological Sciences, Biological Science Bldg., Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E9 (E-mail: larry.wang@ualberta.ca).

Received 28 July 1999; accepted in final form 12 November 1999.

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