The effect of total parenteral nutrition on vasogenic edema development following cold injury in rats

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Total parenteral nutrition (TPN) has been shown to decrease mortality and to increase the rate of recovery in head-injured patients. However, a recent short-term animal experiment has raised concern over the potential enhancement of vasogenic edema by TPN. The experiment described here was undertaken to examine longer-term effects of TPN infusion on vasogenic edema development. Twenty-four rats received an infusion of a TPN solution (35% glucose) or 0.9% saline at 4 ml/kg/hr for 4 or 26 hours following cold injury. In the 4-hour experiment, TPN increased the serum glucose level to 772 ± 57 mg/dl compared to 160 ± 14 mg/dl in the saline-treated animals (p = 0.0001) and increased serum osmolality to 312 ± 3 mOsm/kg compared to 291 ± 3 mOsm/kg in the saline-treated group (p = 0.0006). In the 26-hour experiment, TPN-infused rats were also hyperglycemic and hyperosmotic by 4 hours postinjury and remained hyperglycemic at 26 hours postinjury (serum glucose level 374 ± 97 mg/dl compared to 141 ± 3 mg/dl in saline-treated animals; p = 0.0371). Although by 26 hours the TPN-infused rats appeared hyperosmotic compared to the saline-treated rats, high variability in the TPN group prevented statistical confirmation of this observation (serum osmolality 337 ± 35 mOsm/kg in the TPN group compared to 287 ± 6 mOsm/kg in the saline group).

A three-way analysis of variance with repeated measures was used to analyze the effect of infusion (saline vs. TPN), time (4 vs. 26 hours), and cold injury on the specific gravity of the five brain regions studied. Cold injury significantly increased edema development in the injured versus uninjured hemisphere for every region studied (p ≤ 0.0034, all five regions), and edema development increased significantly between 4 and 26 hours in three of the five regions (p ≤ 0.0207, all three regions). The infusion fluid was not a significant factor in any of the analyses. In conclusion, TPN infusion produced hyperglycemia and hyperosmolality in cold-injured rats but did not enhance vasogenic edema development in any brain region studied.

KEY WORDS • vasogenic edema • total parenteral nutrition • cold injury • rat
chloride and 13 mg/kg xylazine). The tail artery and right jugular vein were cannulated. The arterial catheter was constructed of polyethylene (PE 50) tubing (61 cm) connected to 7.6 cm of No. 10 Teflon tubing with cyanoacrylate adhesive. The arterial catheter was kept filled with heparinized saline (100 U/ml). The venous catheter was constructed of 46 cm of PE 50 tubing. Both catheters were tunneled subcutaneously and exited the dorsal surface of the rat at the level of the forepaws. A rodent jacket was placed on each animal.* The catheters were threaded out of the rodent jacket and through a spring tether. The venous catheter was connected to a swivel unit which maintained the hydraulic continuity of the catheter. The hydraulic portion of the swivel unit was connected to an infusion pump allowing venous infusion of 0.9% saline or TPN. The arterial catheter was plugged and allowed to dangle freely outside the cage for arterial blood sampling.

The rats were then placed in a stereotaxic holder and the dorsal aspect of the skull was exposed. A 5-mm hole was drilled through the skull above the left hemisphere lateral to the sagittal suture and anterior to the coronal suture. With the aid of a surgical microscope, the 4-mm diameter circular surface of a metal rod frozen in liquid nitrogen was placed on the dura for 60 seconds. Infusion of either 0.9% saline or TPN was then initiated (4 ml/kg/hr). The content of 1 liter of TPN solution was: 350 gm dextrose, 35 gm amino acids, 4.5 mEq calcium, 8 mEq magnesium, 40 mEq potassium, 35 mEq sodium, 52.5 mEq acetate, 35 mEq chloride, 12 mM phosphorus, and 8 mEq sulfate. Body temperature was maintained at 37° to 38°C until the rats regained mobility, at which point they were returned to their cage. The swivel unit allowed the rat full range of mobility around his cage while leaving the catheters undisturbed. Rats sacrificed at 4 hours after cold injury needed no food or water; those sacrificed at 26 hours following cold injury were given water ad libitum; food was provided to saline-treated rats only. At the end of the experiment, the rats were reanesthetized with intravenous ketamine (12.5 mg/kg for the 4-hour experiment and 25 mg/kg for the 26-hour experiment) and then were decapitated.

Serum Glucose Level and Osmolality

The serum glucose level and osmolality were measured prior to cold injury, 4 hours after cold injury, and 26 hours following cold injury. To assure the availability of a sample at termination of the experiment, the final blood sample was taken at the time of decapitation. Blood samples were centrifuged and serum was taken for glucose and osmolality measurements. Blood glucose levels were analyzed using the Ames Seralyzer.† Osmolality was measured by the University of Ken-

* Rodent jacket manufactured by Harvard Bioscience, South Natick, Massachusetts.
† Ames Seralyzer manufactured by Miles Inc., Elkhart, Indiana.
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TABLE 1

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Measurements</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preinjury</td>
<td>4 Hrs Postinjury</td>
</tr>
<tr>
<td>4-hour group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>saline</td>
<td>glucose</td>
<td>155 ± 14</td>
</tr>
<tr>
<td>TPN (mg/dl)</td>
<td>142 ± 8</td>
<td>772 ± 57†</td>
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<tr>
<td>saline</td>
<td>osmolality</td>
<td>286 ± 2</td>
</tr>
<tr>
<td>TPN (mOsm/kg)</td>
<td>284 ± 2</td>
<td>312 ± 3†</td>
</tr>
<tr>
<td>26-hour group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>saline</td>
<td>glucose</td>
<td>149 ± 7</td>
</tr>
<tr>
<td>TPN (mg/dl)</td>
<td>158 ± 7</td>
<td>418 ± 45†</td>
</tr>
<tr>
<td>saline</td>
<td>osmolality</td>
<td>273 ± 4</td>
</tr>
<tr>
<td>TPN (mOsm/kg)</td>
<td>281 ± 4</td>
<td>300 ± 3†</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means for six samples each. TPN = total parenteral nutrition.
† Statistically different from saline-treated rats; see text for details.

Results

Physiological Data

Infusion rate and body weight were similar in saline- and TPN-treated rats in both the 4-hour and 26-hour groups. Overall body weight in this study was 323 gm while the average infusion rate for all 24 rats was 4 ml/kg/hr.

The effects of TPN infusion on blood glucose levels and osmolality are shown on Table 1. In the 4-hour group, serum glucose levels and osmolality were similar prior to cold injury and the start of fluid infusion. However, by 4 hours postinjury, the serum glucose content was 772 ± 57 mg/dl in TPN-infused rats compared to 160 ± 14 mg/dl in saline-infused rats (p = 0.0001). Serum osmolality was also increased in TPN-infused rats (312 ± 3 mOsm/kg) compared to saline-infused rats (291 ± 3 mOsm/kg; p = 0.0006).

In the 26-hour group, serum glucose levels and osmolality were also significantly increased at 4 hours in TPN-infused rats (p = 0.0002 and p = 0.0363, respectively), although neither value was as high as was observed in the 4-hour group. By 26 hours postinjury, serum glucose levels were still significantly greater in TPN- versus saline-treated animals (374 ± 97 vs. 141 ± 3 mg/dl; p = 0.0371). Although the TPN-treated rats appeared hyperosmotic compared to the saline-treated rats at 26 hours, the high variability among the TPN-infused animals prevented statistical confirmation of this observation.

Specific-Gravity Data

A three-way ANOVA with repeated measures was used to analyze the specific-gravity data. The data for each region are plotted in Fig. 2. The only significant single factor among all of the regions was the repeated measures factor which examined left to right differences in specific gravity at each brain region studied (p ≤ 0.0034). This observation is clear upon examination of Fig. 2 and demonstrates that cold injury decreased the specific gravity (increased water content) of each region of the injured (left) hemisphere of the brain compared to the unjured (right) hemisphere of the brain.
to the equivalent region of the uninjured (right) hemisphere.

The only significant interaction was the time versus right hemisphere sample/left hemisphere sample interaction. This interaction was significant in the cortex of Slice 1 (p = 0.0004) and in the subcortex of both Slice 2 (p = 0.0138) and Slice 3 (p = 0.0207), and indicates that the cerebral edema development continued between 4 and 26 hours in these regions, increasing the difference in brain water content between the injured and uninjured hemisphere.

The type of infusion a rat received was not significant either as an overall factor or as part of any interaction. Therefore, TPN infusion did not enhance edema formation produced by cold injury compared to saline infusion in any region studied. To test the effects of TPN on the individual hemispheres, unpaired t-tests were conducted between saline- and TPN-treated rats. The TPN infusion did not alter the specific gravity of the right (uninjured) hemisphere in any region at either 4 or 26 hours, and did not alter the specific gravity of the left hemisphere in any region at any time either.

Discussion

The primary finding of this investigation was that a TPN infusion for up to 26 hours produces hyperglycemia and hyperosmolality, but does not enhance edema development in any region studied. Our conclusion that TPN does not increase vasogenic edema in this cold-injury model is in disagreement with the study by Waters, et al.10 In their study, they evaluated vasogenic edema development following cold injury in cats by measuring the volume of EB extravasation and found that a TPN solution similar to ours increased EB extravasation volume. There are a number of possible explanations for this discrepancy. Although the animals in both studies were infused at similar rates with similar TPN solutions, the osmotic challenge was considerably greater in the Waters study. In that study, TPN infusion increased osmolality to 49.9 mOsm/liter. This is considerably more than the 21-mOsm/kg increase in osmolality produced by 4 hours in our study and could reflect more efficient distribution and metabolism of the TPN solution by rats compared to cats. If hyperosmolality can increase vasogenic edema development, the degree of hyperosmolality produced by TPN in our study may not have been severe enough to produce such an effect. In our short-term experiments, the animals were also sacrificed 1 hour later than the animals in the Waters study, but the significance of this factor is not known. By 26 hours postinjury the increase in osmolality in our TPN-infused rats was approximately the same as in the cats in the Waters study (50 mOsm/kg). Although not statistically greater than in the saline-infused rats due to the high variation in the TPN group, this 50-mOsm/kg increase in osmolality was not associated with increased edema development in TPN-infused rats.

Waters, et al.,10 studied white matter edema. The white matter is a preferred low-resistance pathway for edema spread9 and may therefore be more susceptible to osmotic factors. White matter was not specifically sampled in our study. Any specific-gravity changes in white matter may have been masked in our study by specific-gravity changes in the gray matter, which made up the bulk of the tissue sampled.

The quantification of edema was also different in the two studies. We evaluated vasogenic edema on the basis of specific-gravity changes in anatomically defined regions. Waters, et al.,10 measured EB extravasation with regional sampling of EB-stained white matter from coronal slices at varying distances from the frontal pole. By their observation of increased EB volume and similar specific gravities at any one slice, Waters, et al., concluded that their TPN-treated animals showed increased white matter water content. The overriding assumption in reaching this conclusion was that the few samples taken were representative of the water content of the entire EB-stained sample. However, as the data from their study indicate, EB-stained tissue can vary widely in specific gravity. Therefore, whether increased EB extravasation volume represents a true increase in hemispheric tissue water or simply indicates a wider distribution of the same amount of edema is unclear. More data are needed to resolve this issue.

In our study, brain edema development was evaluated by examining the specific gravity of anatomically defined regions which accumulated varying amounts of edema. An increase in the spread of edema from the site of injury would imply increased bulk flow of water into or through these defined regions. In either case, the net water content of such regions would be increased and specific gravity would be decreased. The TPN infusion did not produce any such effect in any region studied. It was concluded, therefore, that TPN does not enhance edema development in the rat model of cold injury despite its hyperosmotic and hyperglycemic qualities.

These data also imply that hyperglycemia is not a critical factor in the development of vasogenic edema in this model. Although hyperglycemia enhances cerebral injury and edema following ischemic insults,5,7-9 the findings of our present TPN study are in agreement with the results of our previous work which indicate that different glycemic states do not alter edema development in this model.4 Since cold injury does not produce ischemia severe enough to cause complete ATP depletion,1 this model of head trauma may not be sensitive to hyperglycemia's deleterious effects. The ability of TPN to cause significant hyperglycemia may be of concern in situations in which cerebral ischemia is present.

Interestingly, the hyperglycemic and hyperosmotic effects of TPN noted in this animal model are partly demonstrable in head-injured patients placed on TPN infusion for up to 18 days.11 Patients receiving TPN within 48 hours after head injury demonstrated a non-
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significant tendency to higher serum osmolality than enterally fed patients over the first 5 days postinjury. The TPN-treated patients also demonstrated a tendency toward higher serum glucose levels over the initial 13 days postinjury compared to enterally fed patients. This trend reversed after Day 13, when the enterally fed patients showed higher glucose levels. Despite these trends, TPN did not significantly enhance intracranial pressure over the 18 days of the study. If intracranial pressure is taken as an indicator of brain edema, these clinical data are in agreement with the experimental data in the present study.

In summary, TPN infusion can cause significant hyperglycemia and hyperosmolality in cold-injured rats. Despite these effects, no evidence was found that TPN increased cerebral edema in this model.

References

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