Role of Endothelin-1 in Diabetic Ketoacidosis

Suresh Havelad, MD, Anil Gulati, MD, PhD, Mansh S. Lavelle, PhD, Katie Koenig, MS

*Adovocate Lutheran General Children's Hospital, Park Ridge, IL, USA and #Midwestern University, Downers Grove, IL, USA

Introduction

Diabetes mellitus (DM) is a major cause for hospital admission in children resulting in 33,888 hospital admissions a year. Approximately 186,300 children in the U.S. have DM and its incidence in children continues to grow in all developed nations. In children with type 1 DM, diabetic ketoacidosis (DKA) is often the first sign of the disease, with up to 20% of newly diabetic children presenting with DKA. Once pancreas replacement with insulin is started, the disease can be controlled, with the morbidity and mortality of DKA reduced. The mechanism for the development of CE is unknown. Endothelin (ET), a twenty-one amino acid vasoconstrictive peptide, displays a widespread range of functions in the body. ET-1 has been found to regulate insulin response, may affect insulin resistance, and increased levels are associated with cerebral vasocconstriction. BMS-182874 has been extensively studied in various animal models and in humans. BMS-182874 has been used to improve the treatment of DKA.

Methods & Materials

Sample
- Male Sprague-Dawley rats weighing 300 to 350 g (Harlan, Indianapolis, IN)

Study Procedures
- Induction of diabetic ketoacidosis (DKA): After 2 hours of fasting, rats were injected IP with 150 mg/kg of Streptozocin (STZ) in 0.05 mol/L citric acid, pH 4.3 to induce diabetes.
- Baseline blood glucose and ketone levels were estimated before STZ injection and then on Days 2 and 3 to ensure the onset of hyperglycemia and ketosis. Significantly elevated urine ketone levels (>160 mg/dL), ketone levels in blood (>20 mg/dL), and blood glucose levels (>300 mg/dL) were estimated on Day 2 to estimate the development of DKA.

Treatment Procedure
- Group 1: Non-diabetic: no treatment
- Group 2: Diabetic: no treatment
- Group 3: Diabetic: saline infusion (0.9% NaCl at 80 mL/kg/hr for 1st hour, then at 40 mL/kg/hr for 2nd to 4th hour)
- Group 4: Diabetic: saline and insulin infusion (0.9% NaCl at 40 mL/kg/hr for 1st hour, then at 20 mL/kg/hr for 2nd to 4th hour, 1.5 U/kg/hr regular insulin)
- Group 5: Diabetic: BMS bolus, (9 mg/kg body weight) plus saline and insulin infusion as above

Determination of blood gases:
The animals were euthanized; brain and lungs were dissected out, rinsed with saline, and weighed (wet weight). The animals were dried at a temperature of 60°C for 72 hours and weighed again (dry weight).

Figure 1: Effect of DKA and Treatment on Mean Arterial Pressure

Figure 2: Endothelin-1 (ET-1) levels Pre and Post treatment. ET-1 levels were higher in study animals with DKA.

Results

Table 1: Effect of DKA and Treatment on Blood Glucose Levels (mg/dL)

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Day 1 (Pre)</th>
<th>Day 2 (Pre)</th>
<th>Day 3 (Pre)</th>
<th>Day 4 (Pre)</th>
<th>Day 5 (Pre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic treated</td>
<td>6.80 ± 0.00*</td>
<td>6.81 ± 0.02*</td>
<td>6.91 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
</tr>
<tr>
<td>D-Keto treated</td>
<td>6.80 ± 0.00*</td>
<td>6.81 ± 0.02*</td>
<td>6.91 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
</tr>
<tr>
<td>D-Saline treated</td>
<td>6.80 ± 0.00*</td>
<td>6.81 ± 0.02*</td>
<td>6.91 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
</tr>
<tr>
<td>D-Saline + insulin</td>
<td>6.80 ± 0.00*</td>
<td>6.81 ± 0.02*</td>
<td>6.91 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
</tr>
<tr>
<td>BMS bolus-treated</td>
<td>6.80 ± 0.00*</td>
<td>6.81 ± 0.02*</td>
<td>6.91 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
<td>6.84 ± 0.02*</td>
</tr>
</tbody>
</table>

Results indicate that induction of DKA by STZ increased blood glucose levels and treatment with insulin produced a significant decrease in blood glucose. This also helped in lowering the significant increase in blood glucose levels observed in untreated DKA-treated rats.

Table 2: Effect of DKA and Treatment on Brain Water Content

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Day 1 (Pre)</th>
<th>Day 2 (Pre)</th>
<th>Day 3 (Pre)</th>
<th>Day 4 (Pre)</th>
<th>Day 5 (Pre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic treated</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
</tr>
<tr>
<td>D-Keto treated</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
</tr>
<tr>
<td>D-Saline treated</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
</tr>
<tr>
<td>D-Saline + insulin</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
</tr>
<tr>
<td>BMS bolus-treated</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
<td>40.2 ± 0.2</td>
</tr>
</tbody>
</table>

The brain water content was significantly (*P<0.05) decreased in study animals with DKA who were treated with insulin. This increase in cerebral perfusion was attenuated when an endothelin-1 antagonist was used. This demonstrates that endothelin treatment is not a cause of increased brain water content but the water content was more likely a result of rehydration rather than cerebral edema.

Figure 3: Endothelin-1 (ET-1) levels Pre and Post treatment. ET-1 levels were higher in study animals with DKA.

Discussion

This study demonstrated an increase in cerebral perfusion in animals with DKA who were treated with insulin. This increase in cerebral perfusion was associated with increased levels of Endothelin-1 levels in animals with DKA. In addition ET-1 levels were higher in animals with DKA and increased with the administration of insulin.

Figure 4: Endothelin-1 (ET-1) levels Pre and Post treatment. ET-1 levels were higher in animals treated with insulin.

Conclusion

- Induction of DKA in study animals is associated with increased levels of Endothelin-1 levels
- Treatment with insulin causes a further increase in Endothelin-1 levels
- A significant increase in brain perfusion following administration of insulin in animals without any significant change in hemodynamic parameters
- ETA receptor antagonist, BMS12074 was found to block this increase in brain perfusion

Figure 5: Effect of DKA and Treatment on Brain Water Content

The brain water content was significantly (*P<0.05) decreased in untreated DKA rats as compared to normal rats. Treatment with saline, salmin+insulin or BMS-treatment increased brain water content but the water content was more likely a result of rehydration rather than cerebral edema.

References