Case Report

Sirolimus-Induced Hepatitis in Two Cases with Hyperinsulinemic Hypoglycemia

Short Title: Hepatitis due to sirolimus in CHI

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What is already known on this topic?
Sirolimus is an alternative for the treatment of congenital hyperinsulinism (CHI) unresponsive to diazoxide and octreotide.

What this study adds?
This is the first report of sirolimus-induced hepatitis in childhood hyperinsulinemic hypoglycemia.

Abstract

Background: Sirolimus has been described for the treatment of the diffuse form of congenital hyperinsulinism (CHI) unresponsive to diazoxide and octreotide without severe side effect.

Case Report: Two newborns with CHI due to homozygous ABCC8 gene mutations were started sirolimus due to unresponsive to medical treatment on day 21 and 17, and good response was observed. At follow-up, liver enzyme levels increased at 10 and 2 month of therapy in case 1 and 2, respectively (serum sirolimus level 1.4ng/ml (N:5-15), AST:298U/L, ALT:302U/L and serum sirolimus level: 9.9ng/ml, AST:261U/L,ALT:275U/L, respectively). In case 1, discontinuation of the drug resulted in normalization of liver enzymes within the next 3 days. Two days after the normalization, sirolimus was restarted with a lower dose, which resulted in an increase again in enzyme levels. In case 2, the reduction of sirolimus dose caused normalization in liver enzymes within 10 days. When the dose was increased, the enzymes were elevated again three days later. Sirolimus was discontinued in both cases.

Discussion: The rapid normalization of liver enzyme levels after sirolimus withdrawal or dose reduction; elevation of transaminases after restart or dose increment and rapid normalization after sirolimus withdrawal again indicated the diagnosis of sirolimus induced hepatitis. To the best of our knowledge, this is the first report of sirolimus-induced hepatitis in CHI. Sirolimus is a promising drug for CHI unresponsive to medical treatment, but physicians should be vigilant for adverse effects.

Keywords: Hyperinsulinemic hypoglycemia, sirolimus, hepatitis, liver enzymes
Background:
Congenital hyperinsulinism (CHI) is characterized by inappropriate insulin secretion despite hypoglycemia. CHI is a heterogeneous disorder and the clinical manifestations range from severe hypoglycemia in the newborn period to mild hypoglycemia in childhood (1,2). The incidence is approximately 1:30,000 live births but is increased in populations with a high prevalence of consanguinity (3). Most cases of CHI are caused by autosomal recessive mutations in the \textit{ABCC8} and \textit{KCNJ11} genes (1).

The treatment of severe diffuse CHI unresponsive to diazoxide and octreotide is subtotal pancreatectomy. This surgery is associated with high incidence of insulin-dependent diabetes, persistent hypoglycemia and exocrine pancreatic insufficiency (4). As a novel agent, the mammalian target of rapamycin (mTOR) inhibitor, sirolimus, has been described for the treatment of the diffuse form of CHI unresponsive to diazoxide and octreotide and also reported as a safe agent in pediatric case reports (5-7). Herein, we report two cases of diazoxide unresponsive CHI due to homozygous \textit{ABCC8} gene mutations in whom sirolimus had to be discontinued because of drug related hepatotoxicity.

Case 1:
A female infant presented with severe hypoglycemia on the first day of life and CHI was diagnosed based on laboratory findings. She was normoglycemic with intravenous (iv) glucose, diazoxide, iv glucagon and octreotide on day 16 but the reduction in glucose requirement was not successful during the next 5 days (Table 1). She had also congenital hypothyroidism with normal thyroid USG (TSH: >100 uIU/ml, sT4: 0.7 ng/dl) and was euthyroid with L-thyroxine (12 mcg/kg/d). 18F-DOPA PET CT (positron emission tomography) scanning could not be performed but sequence analysis identified a novel homozygous p.H59P (c.176A>C) missense mutation in the proband’s \textit{ABCC8} gene. \textit{In silico} analysis predicted the variant was likely to be pathogenic and that the affected residue was highly conserved across species (Alamut, Rouen, France). The identification of a recessively inherited \textit{ABCC8} mutation in the patient was consistent with diffuse pancreatic disease. After consent from the parents was obtained, sirolimus was commenced at a dose of 0.5 mg/m²/day on day 21. The serum level of sirolimus and laboratory tests (full blood count, kidney and liver function tests, lipid profile, electrolytes) were checked every 5 days to maintain therapeutic levels between 5-15 ng/dl. She was discharged on day 72 with oral feeding, subcutaneous octreotide (40 mcg/kg/d) and oral sirolimus (3 mg/m²/day). The sirolimus level and biochemical markers were checked at a monthly interval. Since she was normoglycemic, the octreotide doses were decreased during follow-up. At the age of 10 months the patient presented with diarrhea, at this time she was treated with octreotide (6 mcg/kg/d) and sirolimus (3.1 mg/m²/day) and was normoglycemic. Her laboratory tests revealed elevated liver enzymes (Table 2). The coagulation tests, bilirubin levels, ALP, GGT and abdominal ultrasound were performed, and all of those were normal. Although the sirolimus level was below the therapeutic range (1.4 ng/ml), it was discontinued due to its possible hepatotoxic effect. The liver enzyme levels during dose adjustments were shown in the figure. After sirolimus was ceased, the octreotide dose was increased up to 48 mcg/kg/d to achieve normoglycemia and 4 months later the patient was switched to octreotide-LAR. She is currently 18 months of age with normal neuro-motor development and normoglycemic treated solely with octreotide-LAR (15 mg/monthly, 41 mcg/kg/d) and oral feeding with 3 hours intervals. Last HbA1c is 4.9% (30 mmol/mol).

Case 2:
A female infant was referred to our clinic on day 14 of life with CHI resistant to medical therapy (Table 1) and the reduction in glucose requirement was not successful. She had also congenital hypothyroidism with normal thyroid USG (TSH: >100 uIU/ml, sT4: 0.9 ng/dl) and was euthyroid with L-thyroxine (8 mcg/kg/d). Sequence analysis identified a previously reported homozygous missense mutation, p.A1185E (c.3554C>A), in \textit{ABCC8} (8). The presence of a homozygous mutation in the patient was in keeping with diffuse pancreatic disease. After consent from the parents was obtained, sirolimus (0.5 mg/m²/day) was added due to no reduction in glucose requirement on day 17. Serum level of sirolimus was checked every 5 days to keep a therapeutic serum level (5-15 ng/ml). Clinical or laboratory side
effects were not observed. She was discharged at the age of 40 days with sirolimus 0.4 mg/m²/day and octreotide 23 mcg/kg/d.

One month later, routine blood tests for side effects determined elevated liver enzymes (Table 2) without any clinical symptoms and the sirolimus level was 9.9 ng/ml. Also, other laboratory tests (blood count, kidney function tests, ALP, GGT, bilirubin levels, abdominal ultrasound) revealed normal. The liver enzyme levels during dose adjustments were shown in the figure. As sirolimus was discontinued, the dose of octreotide was increased from 10 to 45 mcg/kg/d. Although, the glucose levels were generally close to the lower limit of normal, with frequent oral feeding and the maximum dose of octreotide, we were able to protect the patient from severe hypoglycemia (glucose <50 mg/dl). Subcutaneous octreotide was switched to octreotide-LAR five months later. She is currently 13 months of age and normoglycemic with octreotide-LAR (15 mg/monthly, 45 mcg/kg/d) and oral feeding with 4 hours intervals. Last HbA1c is 4.2% (22 mmol/mol).

**Discussion:**
The aim of treatment of CHI is to achieve normoglycemia and to prevent neurological damage. However, the clinical management of severe diffuse CHI unresponsive to medical treatment is still a huge problem (4). In a recent study, mTOR inhibitor sirolimus has been described as a novel agent for the treatment of diazoxide unresponsive CHI and achieved normoglycemia with no major adverse effect in 4 cases (5). We now report two further cases with severe CHI due to a homozygous ABCC8 mutation. Both were successfully treated with sirolimus consistent with other reported cases, but sirolimus had to be discontinued because of drug-induced hepatitis.

Various side effects of mTOR inhibitors were reported which are reversible with dose reduction, such as bone marrow suppression, dyslipidemia, immunosuppression, elevation of liver enzymes, renal dysfunction, pneumonitis and stomatitis in adult studies (9,10). In children, this drug was well tolerated in several studies within normal blood level of sirolimus with normal or high doses (1-6 mg/m²/d) (11-13). The main side effect in these studies was oral mucositis. However, in a recent study, Szymanowski al. investigated the efficacy and adverse effect profile of sirolimus in the treatment of severe CHI (14). They detected adverse events in 80% of the patients (hypertriglyceridemia, anemia, stomatitis, sepsis, varicella zoster and gut dysmotility) with 30% therapeutic success.

Hepatotoxicity is another side effect of sirolimus resulting in transient and mild increase in liver enzymes and its incidence was reported to be 17% in patients with renal transplant (15). Seniappean al. and Meder al. reported mild transient elevation of liver enzyme levels and these increments were not more than double the normal range and resolved spontaneously or with reduction in sirolimus dose (5,6). Although, sirolimus seems to be safe in terms of hepatotoxicity, there are case reports with severe sirolimus induced hepatitis. First report was a patient with renal transplantation who received sirolimus as an initial immunosuppressive in post-transplant period (16). At 16th month post-transplant, increased liver enzyme levels were detected (max AST:368 IU/l, ALT:579 IU/l) with 6.3 ng/dl serum sirolimus level. After sirolimus withdrawal, quick normalization of aminotransferases was observed. Jacques et al. reported another case with renal transplantation (17). In the second month of sirolimus, biochemical tests showed acute hepatitis (AST: 861 IU/l, ALT: 609 IU/l) with signs of hepatic insufficiency. The serologic and autoimmune markers for the etiology of hepatitis were normal. Despite normal sirolimus level (10 ng/ml), it was withdrawn and transaminase levels normalized within 5 weeks. In our two cases, after sirolimus was discontinued in one case and decreased in the other, the normalization of transaminases was observed within a few days.

Although, octreotide is usually well tolerated in most patients with CHI, octreotide induced hepatitis has been reported in few patients with CHI (18-21). Hepatitis could appear even if the doses are within normal range but the withdrawal of octreotide result in resolution. The rapid normalization of liver enzyme levels after sirolimus withdrawal and dose reduction for our first and second case respectively; elevation of transaminases after restart or dose increment and rapid normalization after sirolimus withdrawal again while the patient was under octreotide treatment indicated the diagnosis of sirolimus induced hepatitis.

Fortunately, the patients are now normoglycemic with octreotide-LAR and frequent feeding. This observation may suggest that the disease could become milder as the time elapses and therefore fairly good response to octreotide-LAR was seen.
Octreotide may alter the thyroid hormones and cause hypothyroidism with low TSH. Hypothyroidism with elevated TSH was reported in two cases with octreotide treated CHI due to ABCC8 gene mutation (19,20). Similarly, our cases had elevated TSH with low fT4 that is characteristic finding for primary hypothyroidism. This is most probably a coincidental finding since the patients on octreotide therapy develop central hypothyroidism marked by low TSH.

In conclusion, sirolimus is a promising drug for the diazoxide unresponsive CHI, but physicians should be vigilant for adverse effects of sirolimus and withdrawal of the drug is the necessarily.

Contributors’ Statements:
Belma Haliloglu conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.
Heybet Tuzun, Muhittin Celik, Avni Kaya and M.Nuri Ozbek contributed to acquisition and interpretation of data, and approved the final manuscript as submitted.
Sian Ellard and Sarah E.Flanagan carried out the genetic analyses and reviewed the manuscript and approved the final manuscript as submitted.

References:


Table 1: The clinical features of the patients before Sirolimus

<table>
<thead>
<tr>
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<th>Case 1</th>
<th>Case 2</th>
</tr>
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<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Birth weight</td>
<td>3300 gr</td>
<td>3100 gr</td>
</tr>
<tr>
<td>Gestation week</td>
<td>39 wk</td>
<td>36 wk</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
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<td>20</td>
</tr>
<tr>
<td>Insulin (mIU/ml)</td>
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<td>43</td>
</tr>
<tr>
<td>Genetic result</td>
<td>ABCC8 gene</td>
<td>ABCC8 gene</td>
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<tr>
<td>Treatment before Sirolimus</td>
<td></td>
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<tr>
<td>GPR</td>
<td>18 mg/kg/min</td>
<td>11 mg/kg/min</td>
</tr>
<tr>
<td>Dinzoxide</td>
<td>15 mg/kg/d</td>
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<tr>
<td>Octreotide</td>
<td>40 mcg/kg/d</td>
<td>40 mcg/kg/d</td>
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<tr>
<td>Glucagon</td>
<td>0.01 mg/kg/h</td>
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<tr>
<td>Age at Sirolimus treatment</td>
<td>Postnatal 21 day</td>
<td>Postnatal 17 day</td>
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</table>
Table 2: The liver enzyme levels of the patients during hepatotoxic period of Sirolimus.

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>day 0</td>
<td>day 3</td>
</tr>
<tr>
<td>AST (0-40 U/L)</td>
<td>298</td>
<td>68</td>
</tr>
<tr>
<td>ALT (0-40 U/L)</td>
<td>302</td>
<td>140</td>
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<tr>
<td>Sirolimus dose (mg/m2/d)</td>
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</table>

Figure Legend:

Figure: The liver enzyme levels (AST and ALT) of case 1 and case 2 during sirolimus treatment