

CASE REPORT

The value of in vitro studies in a case of neonatal diabetes with a novel Kir6.2-W68G mutation

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Key Clinical Message

In infants, especially with novel previously undescribed mutations of the K_{ATP} channel causing neonatal diabetes, in vitro studies can be used to both predict the response to sulphonylurea treatment and support a second trial of glibenclamide at higher than standard doses if the expected response is not observed.

Keywords

Glibenclamide, in vitro, K-ATP channel, neonatal diabetes.

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Introduction

Nearly half (46%) of patients with neonatal diabetes mellitus (NDM) have a gain-of-function mutation in either the Kir6.2 (*KCNJ11*) or SUR1 (*ABCC8*) subunit of the ATP-sensitive potassium (K_{ATP}) channel [1, 2]. Approximately, 31% of cases are due to mutations in *KCNJ11* and approximately 13% are due to mutations in *ABCC8* [3, 4]. The majority of patients with NDM caused by previously described K_{ATP} mutations respond to sulphonylurea therapy, allowing transition off subcutaneous insulin [5, 6]. Identification of a genetic mutation is now possible within 1 week. However, the response to glibenclamide may be difficult to predict for novel mutations.

Methods

We describe the clinical course of an infant with NDM, with a novel mutation in *KCNJ11* who failed to switch from

insulin to glibenclamide. The initial unexpected lack of response to the standard recommended doses of sulphonylurea led to in vitro studies to guide the treatment with higher doses, resulting in the eventual successful transition off insulin.

A female infant was born at 37 weeks gestation by Caesarean section due to severe intrauterine growth retardation. The birth weight was 1.95 kg (−2.4 SDS) and the infant showed signs of a severe growth restriction but was otherwise well. Initial capillary blood glucose level was normal (74 mg/dL, 4.1 mmol/L). However, by day 6 of life, prefeed and post feed blood glucose levels were elevated (144–396 mg/dL, 8–21.5 mmol/L). “A C-peptide response of 223 pmol/L post feed was demonstrated on day 6 with a corresponding glucose levels of 396 mg/dL, 21.5 mmol/L”.

She was treated with subcutaneous insulin titrated with oral feeds and intravenous dextrose but glycemic control was erratic. After 1 week she was managed with multiple daily doses of basal bolus insulin on full oral feeds.

Results

A novel *de novo* *KCNJ11* missense mutation, p.W68G (c.202T>G), was identified. Patients with other amino acid substitution mutations in the same position, p.W68R (causing transient NDM [7]), and p.W68L (in a patient with permanent neonatal diabetes (PNDM), Ellard, and Hattersley, unpublished data) have successfully transferred from insulin to sulphonylurea treatment. Therefore, we commenced oral glibenclamide on day 20 of life in two divided doses, initially at a dose of 0.2 mg/kg/day, and increased this to 1 mg/kg over 4 weeks according the Exeter protocol for PNDM [8]. This was continued for another 4 weeks, but no response was evident. Continuous subcutaneous insulin infusion by pump was commenced at age 2 months (weight 3 kg). Due to the failed response, further analysis of the C-peptide response was

undertaken, which showed that the initial C-peptide response was no longer detectable (<94 pmol/L).

In vitro studies showed the Kir6.2-W68G mutation reduced K_{ATP} channel inhibition by ATP, thus accounting for the diabetes (Fig. 1A). The mutant channel was also sufficiently sensitive to glibenclamide block (Fig. 1B) and the child would be expected to respond to glibenclamide. In vitro studies indicated that 400 nM glibenclamide blocked mutant channels by $95 \pm 1\%$ ($n = 10$). Comparison with other mutations indicated the mutant channel was sufficiently sensitive to sulphonylureas so it was predicted the patient should be able to transfer to sulphonylurea therapy (Fig. 1C).

Glibenclamide was therefore recommenced at age 9 months at a total daily dose of 1.5 mg/kg/day and increased after 1 week to 2 mg/kg/day. Gastrointestinal side effects were noted from days 2–6 after the dose reached

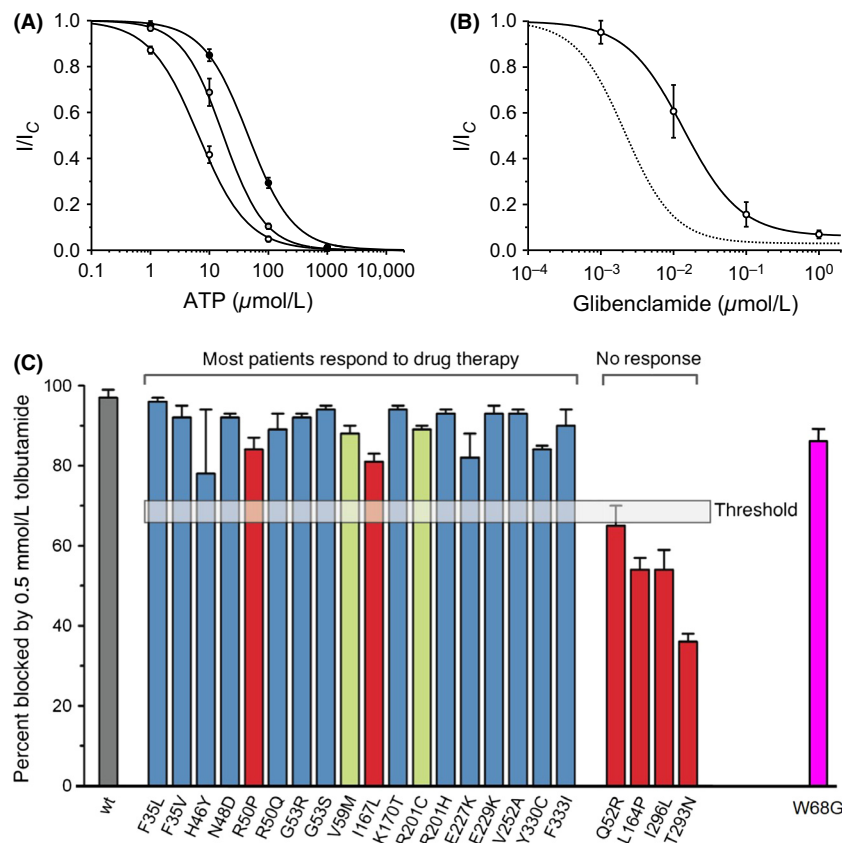


Figure 1. Kir6.2-W68G channels are less blocked by ATP but effectively blocked by glibenclamide. (A) Concentration–inhibition relationships for ATP in Mg-free solution measured for wild-type channels (white circles; $n = 6$; $IC_{50} = 7 \mu\text{mol/L}$), heterozygous channels (gray circles; $n = 7$; $17 \mu\text{mol/L}$), and homomeric Kir6.2-W68G/SUR1 channels (black circles; $n = 6$; $45 \mu\text{mol/L}$). See ref 14 for methodological details [14]. (B) Concentration–inhibition relationships for glibenclamide block of wild-type K_{ATP} channels (dotted line; data taken from [Proks et al., 2013]; $IC_{50} = 2 \text{ nmol/L}$) and Kir6.2-W68G/SUR1 channels in azide-treated oocytes ($n = 10$; $IC_{50} = 14 \text{ nmol/L}$). See ref 14 for methodological details [14]. (C) Extent of block produced by the sulphonylurea tolbutamide for wild-type channels (>95%, far left, wt) and K_{ATP} channels carrying different mutations in Kir6.2 (as indicated). The gray bar indicates a crucial threshold: most people who have a mutation that lies above the threshold can transfer to sulphonylurea therapy. None of those with mutations that lie below the line can transfer. Our patient with a Kir6.2-W68G mutation (in pink) clearly lies above the line indicating that they should respond to sulphonylurea therapy.

2 mg/kg/day. After 1 week of glibenclamide at 2 mg/kg/day, frequent episodes of hypoglycemia were noted requiring a dramatic reduction in insulin. Plasma levels of glibenclamide at different doses were measured by liquid chromatography–mass spectrometry (LC–MS) analysis [9] immediately before dosing, when drug levels were expected to be at their lowest. The drug concentration was 77.3 ± 3.9 nmol/L on a dose of 1 mg/kg/day and increased to 111.8 ± 2.8 with 1.5 mg/kg/day and to 355.0 ± 13.4 nmol/L on 2 mg/kg/day. A clinical response was only achieved at the higher plasma glibenclamide, indicating the lower doses were subtherapeutic in this patient.

After a week of dose adjustments a three times daily dosing of glibenclamide (1.85 mg/kg/day) was found to be the most effective, to avoid hypoglycemia and maintain a good glycemic control.

The patient has successfully transferred off insulin and a C-peptide response has been demonstrated again. An improved glycated hemoglobin A1c (HbA1c) (8%, 64 mmol/mol pre transfer; 6.8%, 51 mmol/mol post transfer; normal range 20–42), along with a better weight gain, was noted within 6 weeks. Weight and height are on the 9th centile. She has excellent neurodevelopment at 16 months and her HbA1c has almost normalized (6.1%, 43 mmol/mol) with no significant hypoglycemia. The current dose of glibenclamide is 1.6 mg/kg/day.

Discussion

This patient, with a novel *KCNJ11* mutation, failed to respond to the standard dose of 1 mg/kg/day of glibenclamide as currently recommended, but has successfully responded to 2 mg/kg/day. She is now maintained on 1.6 mg/kg/day with excellent glycemic control. In vitro studies indicated that the child should respond that prompted a retreat of glibenclamide therapy and analysis of plasma glibenclamide levels. The initial failure to respond was attributable to subtherapeutic glibenclamide levels in this patient on the standard doses. The reason as to why this initial failure to respond is unclear, but may be a function of her specific mutation, or due to other effects.

Recommendations are that all patients diagnosed with PNDM in the first 6 months should be tested for *KCNJ11* mutations [10]. In addition, we have demonstrated that the knowledge of previously described mutations and their response to sulphonylureas can be useful in guiding the treatment.

Most NDM patients (>90%) have been transferred to sulphonylurea therapy while receiving very high doses of sulphonylureas, equivalent to 0.8 mg/kg/day for at least 4 weeks [6]. The median dose is 0.5 mg/kg and the usual range is 0.13–1.2 mg/kg [5]. These doses are much higher than the doses used for type 2 diabetes. Once the initial

glycemic control is achieved in NDM, control may improve with time and the sulphonylurea dose reduces, which was also our experience.

Successful treatment of NDM with sulphonylurea doses up to 2 mg/kg/day has been reported previously, and higher doses are generally required for patients with *KCNJ11* mutations compared with patients with *ABCC8* mutations [11]. Higher doses are not recommended unless there is a clear reduction in the insulin dose after 4 weeks of 1 mg/kg/day glibenclamide [8]. Children aged under 12 months at transfer often require doses >1 mg/kg to transfer as an inpatient and then may develop hypoglycemia a needing rapid reduction in doses after coming off insulin, as in our patient [6]. This probably relates to the altered renal clearance of sulphonylureas in infants resulting in an increased half life. It may also relate to an improved beta-cell function when improved glycemic control is established [12].

Trying a higher dose was indicated by the in vitro studies in which the mutant channels showed a response to glibenclamide. This example highlights the perils of relying on a trial of sulphonylurea therapy before genetic testing in this condition, which has recently been reported [13].

This example of personalized medicine leading to an improved treatment, glycemic control, and quality of life supports further exploration of the underlying pathophysiology in other conditions, from bench to bedside and back again.

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Conflict of Interest

The authors have no disclosures. S.M.O'C is the guarantor for the contents of this article. S.E and A.T.H are Wellcome Trust Senior Investigators. ATH is an NIHR Senior Investigator and is employed as a core member of the Exeter NIHR Clinical Research Facility. F.M.A holds a Royal Society/Wolfson merit award.

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