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Permanent neonatal diabetes: combining sulfonylureas with insulin may be an effective treatment

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What's new?

- *KCNJ11* mutations causing permanent neonatal diabetes are treated with sulfonylureas, but not all individuals are able to transfer completely from insulin to sulfonylureas.
- Our data highlight that combining sulfonylurea treatment with insulin in those who are unable to fully transfer may still lead to clinically meaningful outcomes.
- We demonstrate improvements in endogenous insulin production, HbA_{1c}, glycaemic variability and hypoglycaemia awareness.
- These changes were not observed at initial doses of glibenclamide and improvements required higher sustained doses of glibenclamide.
- Combining insulin and sulfonylureas should be considered in those with permanent neonatal diabetes who are not able to transfer to sulfonylurea therapy alone.

Abstract

Background Permanent neonatal diabetes caused by mutations in the *KCNJ11* gene may be managed with high-dose sulfonylureas. Complete transfer to sulfonylureas is not successful in all cases and can result in insulin monotherapy. In such cases, the outcomes of combining sulfonylureas with insulin have not been fully explored. We present the case of a woman with diabetes due to a *KCNJ11* mutation, in whom combination therapy led to clinically meaningful improvements.

Case A 22-year-old woman was found to have a *KCNJ11* mutation (G334V) following diagnosis with diabetes at 3 weeks. She was treated with insulin-pump therapy, had hypoglycaemia unawareness and suboptimal glycaemic control. We assessed the *in vitro* response of the mutant channel to tolbutamide in *Xenopus* oocytes and undertook sulfonylurea dose-titration with C-peptide assessment and continuous glucose monitoring.

In vitro studies predicted the G334V mutation would be sensitive to sulfonylurea therapy [91 ± 2% block ($n = 6$) with 0.5 mM tolbutamide]. C-peptide increased following a glibenclamide test dose (from 5 to 410 pmol/l). Glibenclamide dose-titration was undertaken: a lower glibenclamide dose did not reduce blood glucose levels, but at 1.2 mg/kg/day insulin delivery was reduced to 0.1 units/h. However, when insulin was stopped, hyperglycaemia ensued. Glibenclamide was further increased (2 mg/kg/day), but once-daily long-acting insulin was still required to maintain glycaemia. This resulted in improved HbA_{1c} of 52 mmol/mol (6.9%), restoration of hypoglycaemia awareness and reduced glycaemic variability.

Conclusion In people with *KCNJ11* mutations causing permanent neonatal diabetes, and where complete transfer is not possible, consideration should be given to dual insulin and sulfonylurea therapy.

<H1>Introduction

Permanent neonatal diabetes mellitus is a rare monogenic form of diabetes that presents within the first 6 months of life [1]. Mutations in the *KCNJ11* and *ABCC8* genes, encoding the Kir6.2 and SUR1 subunits, respectively, of the pancreatic β-cell ATP-sensitive potassium channel (K_{ATP} channel) account for 50% of cases [2,3]. The K_{ATP} channel plays a key role in insulin secretion by regulating the β-cell plasma membrane potential, and thereby insulin secretion, in response to the ambient glucose concentration [4]. At low glucose levels, the channel is open, keeping the membrane hyperpolarized and inhibiting insulin release. However, when glucose levels rise, the accompanying increase in metabolically generated ATP closes the K_{ATP} channel, resulting in membrane depolarization and activation of voltage-gated calcium entry, which stimulates insulin secretion. Activating mutations in either *KCNJ11* or *ABCC8* impair the ability of the channel to respond to ATP and as a

consequence, the K_{ATP} channel remains open despite of elevated plasma glucose. This impairs insulin release and results in diabetes.

Sulfonylurea drugs bind to the SUR1 subunit of the K_{ATP} channel and close it independently of ATP, thereby stimulating insulin release. They are now the therapy of choice in children with permanent neonatal diabetes caused by mutations in *KCNJ11* or *ABCC8* [5]. Prior to the discovery of the genetic cause of permanent neonatal diabetes, people with the condition were treated with insulin. However, ~ 90% of people with neonatal diabetes due to a *KCNJ11* mutation have been able to successfully transfer from insulin to high-dose oral sulfonylurea therapy, which usually results in significant improvements in HbA_{1c} [5–7].

Unsuccessful transfer is determined by two factors: the sensitivity of the mutant K_{ATP} channel to sulfonylurea inhibition, and the duration of diabetes prior to transfer [6,7]. Some mutant channels are less sensitive to sulfonylurea inhibition, and no cases with a mutation that shows < 60% block of the K_{ATP} current by 0.5 mM tolbutamide in *in vitro* studies, have been able to transfer. In other cases, however, where some people have been able to transfer but others with the same mutation have been unable to do so, the ability to transfer is correlated with the duration of diabetes; an earlier age at transfer is more likely to result in success [6,7].

In those who have been unable to transfer completely to sulfonylureas, variable practice is observed with some reverting to insulin monotherapy, and others remaining on a combination of sulfonylureas and insulin or other agents [6–8]. The clinical benefits of remaining on combined insulin and sulfonylurea therapy have not been fully explored.

We report a case in which sulfonylurea therapy in combination with insulin treatment enabled a significant reduction in insulin dose, transition off insulin pump therapy and led to clinically meaningful improvements in HbA_{1c}, glycaemic variability and hypoglycaemia awareness. We undertook clinical and *in vitro* studies to investigate this phenotype further

and show that the inability to transfer off insulin completely should not be considered a failure if the addition of sulfonylureas to the treatment regime results in improved glycaemia.

<H1>Methods

<H2>Molecular genetics

Genomic DNA was extracted from peripheral leukocytes using standard procedures. The coding regions and conserved splice sites of the *ABCC8* and *KCNJ11* genes were amplified by polymerase chain reaction (PCR) and the resulting amplicons sequenced using the Big Dye Terminator Cycler Sequencing Kit v3.1 (Applied Biosystems, Warrington, UK). The products were analysed on an ABI 3730 capillary sequencer (Applied Biosystems) and compared to the reference sequences (NM_000525.3 and NM_000352.3) using Mutation Surveyor v3.24 software (SoftGenetics, State College, PA, USA).

<H2>Functional studies

We used human Kir6.2 (GenBank NM000525, with E23 and I337) and rat SUR1 (GenBank L40624). Site-directed mutagenesis of Kir6.2 was performed as described previously [6,9]. Defolliculated *Xenopus laevis* oocytes were injected with 0.8 ng wild-type (or mutant) Kir6.2 mRNA and 4 ng SUR1 mRNA, and were incubated in Barth's solution at 18 °C for 1–4 days. To simulate the heterozygous state of the heterozygous phenotype, we co-injected a 1 : 1 mixture of mutant and wild-type Kir6.2, together with SUR1. The resulting channel population (referred to here as hetG334V) contains a variable number of mutant subunits (between zero and four) in the Kir6.2 tetramer.

<H2>Clinical investigations

All studies were undertaken on the clinical investigation unit. Sulfonylurea test dose and oral glucose tolerance tests were undertaken according to the Exeter team protocol [10]. Briefly, following cessation of bolus insulin and a 75-g oral glucose tolerance test, a test dose of

~ 0.1 mg/kg of glibenclamide was administered. Subsequent dose titration was carried out according to the transfer protocol.

<H1>Results

<H2>Clinical data

A 22-year-old woman of South East Asian ethnicity was diagnosed with diabetes at the age of 3 weeks in her home country. This was assumed to be Type 1 diabetes. She was delivered following a caesarean section (breech position) at term weighing 3.2 kg. She developed normally during childhood with no delay in achieving milestones, no epilepsy and only a diagnosis of mild dyslexia. She was treated for 17 years with multiple daily injections of insulin, before commencing insulin pump therapy at the age of 18 years. She had several documented episodes of diabetic ketoacidosis during her childhood and teenage years. Neither of her parents or her only sibling was affected with diabetes.

<H2>Genetic data

Having moved to the UK, genetic testing for permanent neonatal diabetes was requested on the basis of her diagnosis before 6 months of age. Genetic testing revealed a heterozygous *KCNJ11* missense mutation, p.G334V (c.1001G > T), presumed to have occurred *de novo*, given absence of diabetes in either parent.

At the time of genetic testing, the woman had an above target HbA_{1c} of 88 mmol/mol, impaired awareness of hypoglycaemia, background retinopathy and significant abdominal lipohypertrophy (see Table 1 for baseline characteristics). There was no evidence of nephropathy or neuropathy. She required between 80 and 85 units of insulin per day via a pump, delivered with variable basal rates and bolus mealtime insulin. Continuous glucose monitoring (CGM) was undertaken in view of her frequent hypoglycaemia and revealed significant glycaemic variability (SD 4.0 mmol/l) with hyperglycaemic excursions and frequent hypoglycaemia.

Functional studies (see below) were undertaken to determine if the Kir6.2-G334V mutant channels were sensitive to sulfonylurea inhibition, with positive results. Consequently, a glibenclamide test dose was undertaken following a day admission, as part of clinical care (see Fig. 1). This revealed an immediate and significant rise in C-peptide following a 5 mg glibenclamide test dose, indicating that the β cells were capable of releasing endogenous insulin. A formal glibenclamide trial was therefore undertaken using the Exeter protocol [10] (see Fig. 2 for a summary of the dose titration), and insulin basal rates were reduced in parallel with increasing glibenclamide doses.

Within 8 weeks of commencing treatment, she was receiving a glibenclamide dose of 20 mg twice daily (0.67 mg/kg/day; 40 mg/day total) and her basal insulin (insulin glargine, Lantus) requirements had reduced to a total basal dose of 9.6 units/day via her insulin pump (0.4 units/h). Her median blood glucose, as assessed by frequent home blood glucose monitoring, was 8.4 mmol/l. Basal insulin was further reduced, but glucose levels rose to a median of 12 mmol/l. Glibenclamide was therefore increased to a dose of 70 mg/day (1.2 mg/kg/day in three divided doses of 30, 10 and 30 mg) and basal insulin was reduced to 0.1 units/h (total basal dose 2.4 units/day). At this point, her median blood glucose was 8.6 mmol/l and an attempt to stop insulin completely was undertaken. However, following disconnection of the pump, glucose levels rapidly rose to between 20 and 25 mmol/l within 6 h (capillary blood ketones were 0.4 mmol/l), insulin was therefore recommenced the following day.

Following discussion, a higher dose of glibenclamide was given (40 mg three times daily; 2 mg/kg/day) for several months, however, insulin was still required to maintain normoglycaemia. This was achieved through once-daily injections of long-acting insulin, enabling discontinuation of pump therapy. After commencement of glibenclamide, her HbA_{1c} fell to 52 mmol/mol (6.9%) and her random C-peptide was 637 pmol/l (Table 1). Her

hypoglycaemia awareness was also restored. Repeat CGM revealed some hypoglycaemia and her glargine dose was reduced accordingly. The standard deviation (SD) of blood glucose measurements was significantly lower (at 2.7 mmol/l) than before sulfonylurea therapy. She is currently maintained on 40 mg glibenclamide three times daily and 10 units insulin glargine once daily, with no mealtime insulin bolus.

<H2>Functional studies

The latest crystal structure reveals that residue G334 forms part of the ATP-binding site and may reduce the ability of ATP to bind to Kir6.2, either by steric interference or electrostatic repulsion [11]. We therefore first examined the sensitivity of wild-type (WT) and mutant channels to inhibition by MgATP. Figure S1 shows that WT channels were half maximally blocked (IC_{50}) by 20 ± 1 µM MgATP ($n = 7$). The ATP sensitivity of heterozygous (hetG334V) Kir6.2-G334V/SUR1 channels was significantly decreased ($IC_{50} = 163 \pm 42$ µM, $n = 6$; $P < 0.01$ vs. WT) and that of homozygous (homG334V) channels was reduced even further ($IC_{50} = 2.6 \pm 0.5$ mM, $n = 3$). There was also a substantial increase in the current amplitude at physiological levels of MgATP: at 3 mM, the percentage of unblocked current was $1.2 \pm 0.2\%$ ($n = 7$) for WT, whereas it was $12 \pm 2\%$ ($n = 6$) and $48 \pm 4\%$ ($n = 3$) for hetG334V and homG334V, respectively. This increase in current can account for the diabetes in the presented case and demonstrates that the mutation is pathological.

We next analysed the effects of the Kir6.2-G334V mutation on the metabolic regulation and sulfonylurea sensitivity of the K_{ATP} channel by measuring whole-cell currents. When WT K_{ATP} channels are expressed in *Xenopus* oocytes they are normally closed, due to the high intracellular ATP concentration ($[ATP]_i$). However, they can be opened by lowering $[ATP]_i$ using a metabolic inhibitor such as Na-azide (Fig. 3a). Mutations that reduce the channel ATP sensitivity normally increase the whole-cell current in control solution, reflecting the fact that they are less blocked by resting $[ATP]_i$. Both hetG334V and homG334V currents

were substantially larger than WT both in control solution (Fig. 3a), as expected from their lower ATP sensitivity. The sulfonylurea tolbutamide, at a dose that maximally inhibits the WT channels (0.5 mM) blocked all three types of channel: by 97.2 ± 0.3% (n = 10) for WT, 91 ± 2% (n = 6) for hetG334V and 78 ± 2% (n = 7) for homG334V (Fig. 3b). These results predict that the individual should be sensitive to sulfonylurea therapy.

<H1>Discussion

We report a young woman with a *KCNJ11*-G334V mutation who, despite being unable to stop insulin completely, showed a significant improvement in glycaemic control when high-dose sulfonylurea therapy was combined with insulin. It is noteworthy that this combination therapy also led to a marked improvement in glycaemic variability and in hypoglycaemia awareness. Furthermore, mealtime bolus insulin was no longer required.

The G334V mutation has been described in one previous case, a male, in whom complete transfer was also attempted, around 20 years post diagnosis [8]. Despite a rise in C-peptide concentration, insulin had to be recommenced after a 5-day trial of glibenclamide. The increase in C-peptide demonstrates that β cells were at least partially functional following K_{ATP} channel closure. Based on our results, it seems possible that the reported case could also have been managed on a combination of a low-dose insulin supplemented with sulfonylurea treatment. It is important to titrate sulfonylurea doses gradually over time to ascertain responsiveness, as demonstrated in our case, where starting doses of glibenclamide had little effect on glycaemia.

In vitro functional studies revealed the G334V mutation led to a small reduction in sulfonylurea sensitivity. However, the extent of block (91%) was well within the range predicted to enable complete transfer off insulin, based on previous findings (60–75%) [4,6]. That our case was unable to do so likely reflects the late age at transfer, because transfer success rate declines with duration of diabetes [6,7]. Transfer is unsuccessful in ~ 30% of

those over the age of 18, compared with < 5% individuals with the same mutation who are aged under 2 years. Rodent models suggest one reason for a better outcome when sulfonylurea is commenced early may be that β-cell exposure to chronic hyperglycaemia leads to loss of insulin content, impaired β-cell metabolism-secretion coupling and reduced β-cell mass [12]. Although it is possible that with further titration of glibenclamide, transition off insulin may be achieved, the 3-month trial at higher doses yielded no further reduction in insulin. Stopping insulin was characterized by fasting hyperglycaemia and so insulin was recommenced.

Sulfonylurea therapy led to a reduction in glycaemic variability and abolished the requirement for mealtime insulin. It is likely that both these effects result from restoration of the incretin response. Incretins are only effective when the K_{ATP} channels are closed, which leads to membrane depolarization, calcium influx and insulin release [13]. Thus, incretin-based drugs maybe of greater benefit when combined with sulfonylurea therapy.

It is possible that combination therapy would also be of benefit in people with mutations that have shown a reduced blockade in response to tolbutamide. Further studies are needed to explore this possibility.

Two studies have reported treatment outcomes on numbers of individuals with permanent neonatal diabetes. Thurber *et al*. [7] reported that 2 of 57 individuals remained on both insulin and sulfonylurea therapy. Babiker *et al*. [6], defined successful transfer as the ability to stop insulin completely. Our study suggests that a reduction in insulin dose should also be considered a successful outcome as it is associated with clinical benefits. Indeed, although no clinical outcomes were reported, Babiker *et al*. also recommended trialling combination therapy.

In conclusion, our data show that in people with *KNCJ11* mutations who are unable to transfer completely to sulfonylurea therapy, a combination of insulin and sulfonylurea treatment may be of clinical benefit, improving glycaemic control, hypoglycaemia awareness and glycaemic variability. Furthermore, there may be improvements in quality of life, for example, by cessation of insulin pump therapy and managing mealtimes without bolus insulin. Thus, the inability to fully transfer off insulin should be still considered a valuable treatment in combination with sulfonylureas.

<H1>Addendum

While this paper was in review, a paper appeared reporting that two individuals who were initially managed solely on sulfonylurea therapy failed to retain good glycaemic control after several years, and in these people combination therapy did not produce better glycaemic control [14]. This contrasts with our individual who showed an improved outcome and may be related to diabetes duration or other factors.

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Competing interests

None declared.

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Author contributions

FMA and NSO are the guarantors of this article. SM and NSO collected and analysed the clinical data. EDF undertook genetic testing. NV and EC collected and analysed the TEVC data, NV collected and analysed the patch-clamp data. SM, NV, FMA, and NSO wrote the manuscript. All authors critically reviewed and revised the manuscript and approved the final version.

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FIGURE 1. C-peptide (grey line), insulin (dash line) and glucose (black line) levels during an oral glucose tolerance test (OGTT). Glucose was given at time zero. A dose of 5 mg glibenclamide plus 15 units insulin was given at t = 150 min with lunch. Note the marked increase in C-peptide after glibenclamide.

FIGURE 2. Changes in glibenclamide dose (grey line), median blood glucose (black line) and daily basal insulin (dashed line) dose during glibenclamide dose titration. Note changes in glycaemia with higher doses of glibenclamide not observed at lower doses.

FIGURE 3. Metabolic sensitivity of the wild-type and mutant K_{ATP} channel. (a) Whole-cell currents were measured in oocytes expressing wild-type (WT; n = 10), heteromeric (hetG334V; n = 7) or homomeric (homG334V; n = 6) K_{ATP} channels. The currents were recorded in control solution (black bars), after metabolic inhibition with 3 mM sodium azide (white bars) and after the further addition of 0.5 mM tolbutamide (tolb, grey bars). (b) Mean ± SEM tolbutamide block for WT (n = 10) and hetG334V (n = 6) channels, compared with the mean value for permanent neonatal diabetes mutant channels (n = 19 mutations). Tolbutamide block is expressed as the percentage block of the azide-induced current.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The G334V mutation reduces ATP inhibition of the K_{ATP} channel. MgATP concentration–response relations for wild-type (WT, ■), homomeric G334V (homG334V, ●) and heteromeric (hetG334V, ○) K_{ATP} channels. The curves are the best fit to the Hill equation.

Table 1. Clinical and biochemical data before and after commencement of sulphonylurea therapy

	Before	After
HbA_{1c} mmol/mol (%)	88 (10.2)	52 (6.9)
Random C-peptide (pmol/l)	f	637
Paired random glucose (mmol/l)	9.3	6.9
Total insulin dose in 24 h (units)	80–85	10
Glibenclamide dose	–	40 mg three times daily (2 mg/kg/day)
Weight (kg)	59	53