Acetazolamide efficacy in hypokalemic periodic paralysis and the predictive role of genotype

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Abstract

Objectives: Acetazolamide has been the most commonly used treatment for hypokalemic periodic paralysis since 1968. However, its mechanism of efficacy is not fully understood and it is unknown if therapy response relates to genotype. We undertook a clinical and genetic study to evaluate the response rate of patients treated with acetazolamide and to investigate possible correlations between response and genotype. Methods: We identified a total of 74 genotyped patients for this study. This included patients referred over a 15 year period to the only UK referral center or to a Chinese center and who underwent extensive clinical evaluation. For all genotyped patients the response to acetazolamide therapy in terms of attack frequency and severity was documented. Direct DNA sequencing of CACNA1S and SCN4A was performed. Results: Only 46% of the total patient cohort (34/74) reported benefit from acetazolamide. There was a greater chance of benefit in patients with mutations in CACNA1S (31 responded/55 total) compared to those with mutations in SCN4A (3 responded/19 total). Patients with mutations that resulted in amino acids being substituted by glycine in either gene were least likely to report benefit. Conclusions: This retrospective study indicates that only approximately 50% of genotyped hypokalemic periodic paralysis patients respond to acetazolamide. We found evidence supporting a relationship between genotype and treatment response. Prospective randomized controlled trials are required to further evaluate this relationship. Development of alternative therapies is required.
Introduction

Hypokalemic periodic paralysis (hypoPP) is an autosomal dominant neuromuscular disorder characterised by episodes of flaccid skeletal muscle paralysis accompanied by reduced serum potassium levels. It is caused by mutations in one of two sarcolemmal ion channel genes, \textit{CACNA1S} and \textit{SCN4A}\textsuperscript{1-3} that lead to dysfunction of the dihydropyridine receptor or the alpha sub-unit of the skeletal muscle voltage gated sodium channel Nav1.4. Seventy to eighty percent of cases are caused by mutations of \textit{CACNA1S} and ten percent by mutations of \textit{SCN4A}\textsuperscript{4}.

Acetazolamide has been the most common treatment choice for hypokalemic periodic paralysis for nearly 50 years\textsuperscript{5} although there is no randomised controlled trial data to evidence its efficacy. Since the discovery of the two causative genes it has been occasionally reported that some patients with \textit{SCN4A} mutations associated with hypoPP reacted adversely to acetazolamide but other reports have indicated benefit\textsuperscript{6}. However, there have been no larger scale prospective or retrospective studies evaluating possible relationships between genotype and treatment response. In this study we have had the opportunity to personally assess the relationship between acetazolamide treatment response and genotype in 18 genotyped cases and to relate this to all 56 published genotyped cases. These retrospective data indicate that around 50% of patients have no useful response to acetazolamide and the genotype is an important factor in treatment response.

Methods

Genetic Analysis
Two groups of patients under the authors’ personal care were assessed (cohort 1 and 2). All patients referred to our UK referral center with a possible diagnosis of hypoPP underwent diagnostic genetic testing. We undertook direct automated sequencing of the S4 voltage sensor segments of $\text{CACNA1S}$ and $\text{SCN4A}$ (exons 4, 11, 21 and 30 of $\text{CACNA1S}$ and exons 5, 12, 13, 18, and 24 of $\text{SCN4A}$) as previously described$^4$.

**Standard Protocol Approvals, Registrations, and Patient Consents**

All patients gave written informed consent for the DNA analysis in this study. Ethics committee approval for review of medical records of patients already under our existing care was obtained from the National Hospital for Neurology and Neurosurgery & Institute of Neurology Joint Research Ethics Committee. No intervention was taken beyond the usual clinical care.

**Assessment of treatment response in cohorts 1 and 2**

Only those patients in whom a genetic diagnosis of hypoPP had been confirmed, who had been under our follow up for at least one year and treated with acetazolamide were included in the study. Patients underwent full clinical evaluation on a six monthly or once yearly basis. A detailed retrospective analysis was undertaken of the clinical records of 14 patients at the UK national reference center for muscle channelopathies neuromuscular clinic (cohort 1). Documented frequency, severity and duration of paralytic attacks before and after therapy with acetazolamide was analysed to ascertain response as either beneficial, detrimental or no change (Table 1). A similar survey was undertaken of 4 further patients at a Chinese center in Hangzhou (cohort 2).
Analysis of published cases (cohort 3)

We conducted a PubMed search using the key terms hypokalemic periodic paralysis, periodic paralysis, acetazolamide and carbonic anhydrase inhibitors. Only articles published in English since 1994 (when the genetic basis of hypoPP was identified) were considered. Only cases of genetically confirmed hypokalemic periodic paralysis were selected (cohort 3). Articles meeting these criteria were reviewed and any reported treatment and treatment response were recorded (see supplemental data Table e-1). This data was then used to evaluate the response to acetazolamide by genotype for 56 patients (Table 2). Genotyped patients who had received acetazolamide in isolation or with other therapies (spironolactone or potassium supplements etc) were selected. In an attempt to avoid any bias the analysis of each of the three cohorts was conducted independently by a separate author. A beneficial response was defined as a reduction in frequency, severity or duration of attacks of paralysis.

Results

In cohort 1 57% of patients with CACNA1S mutations which also represented the cohort as a whole reported a beneficial response to acetazolamide therapy. The positive response rate increased to 62% when only patients with the most common HypoPP mutations (R528H, R1239H) were considered. Cohort 2 was considered too small to analyse the results in the same way as the larger cohorts (cohorts 1 and 3) but the overall trend in therapy response was similar i.e. both the CACNA1S Chinese cases (1 R528H and 1 R1239H) but none of the 2 SCN4A cases (2 R672H) benefited from acetazolamide. In
cohort 3 54% of patients with a CACNA1S mutation reported a beneficial response, compared with only 18% of those with an SCN4A mutation. For cohort 3 overall 43% of patients reported benefit. However, if the R528H and R1239H group in cohort 3 were considered alone, the benefit improved to 59%.

If the combined results from all three cohorts are considered: 31 of 55 (56%) CACNA1S patients benefitted from acetazolamide compared to only 3 of 19 (16%) SCN4A patients (p< 0.002 by a chi-square statistic). Hence, a patient with a CACNA1S mutation is 6.9 times as likely to benefit from acetazolamide therapy, than a patient with an SCN4A mutation (95% confidence interval is 1.6 to 26.4).

These findings are consistent with previous reports that there is a greater chance of a deleterious effect or no beneficial effect from acetazolamide in those patients with hypoPP due to SCN4A mutations but that this does not apply to all such patients. Further evaluation of the relationship between precise genotype and treatment response was undertaken using the larger cohorts 1 and 3. We observed in both cohorts that when one of the arginine residues located at the extracellular side of the sarcolemma (see Fig 1) was substituted for a glycine residue (R528G, R1239G, R672G), none of these patients (0/9) reported a beneficial response to acetazolamide, and there was often a deleterious effect although the number of patients with these substitutions was relatively small.

Substitutions of the more inwardly placed arginine at position R675 in SCN4A have been described. Patients with these substitutions (R675G/Q/W) who received acetazolamide therapy were identified in each cohort but were not included in the final analysis as the phenotype has been reported as potassium sensitive normokalemic periodic paralysis and not hypokalemic periodic paralysis7.
Discussion

There are no consensus guidelines for the treatment of hypoPP. Current pharmacological agents commonly used include potassium supplements, potassium sparing diuretics and carbonic anhydrase inhibitors (acetazolamide and dichlorphenamide). Dichlorphenamide is the only therapy for hypoPP to have undergone a randomised double blind placebo controlled cross over trial. This trial showed a significant efficacy of dichlorphenamide in reducing attack frequency but the inclusion criteria were based on clinical diagnosis of hypoPP and not genetic confirmation\textsuperscript{8,9}. A second randomised controlled trial of the efficacy of dichlorphenamide in genotyped cases of both hypo and hyperkalemic periodic paralysis is currently open (see www.clinicaltrials.gov). Aside from this there is very little trial evidence to support the use of any treatment in hypoPP and no randomised controlled trial evidence supporting the most common choice; acetazolamide.

The data presented here suggest that at least half the patients treated with acetazolamide do not get a satisfactory response. A tentative correlation between detrimental response and genotype does emerge and those patients with substitutions to glycine of arginine residues situated towards the extracellular side of the voltage sensors of either Cav1.1 or Nav1.4 (see Fig 1) may be predicted not to respond to acetazolamide. This observation is particularly noteworthy in light of experimental data indicating that the deleterious effects of the R672G substitution on channel gating are insensitive to reductions in pH in vitro. This is in contrast to the deleterious effects of the R669H mutation which were ameliorated by an acidic pH. These in vitro observations predict that such patients would not respond to acetazolamide which produces a metabolic acidosis\textsuperscript{10}. The deleterious
effects studied relate to the ion selective alpha pore. More recent studies have identified an anomalous proton selective gating pore due to R669H and R672H SCN4A mutations and a less selective cation conducting pore in the R672G SCN4A mutation\textsuperscript{11,12}. Similar gating pores have yet to be identified in the \textit{CACNA1S} mutations but are proposed as a likely pathomechanism. This may additionally suggest that the R to H substitutions are likely to have a greater effect on pH concentration gradients and as a result more likelihood of a beneficial response to acetazolamide therapy compared to the R to G substitutions. Overall these data support the view that it is important to achieve a genetic diagnosis in each patient to help guide treatment.

Without randomised controlled trials the exact efficacy of acetazolamide is unknown. In addition its mechanism of action is unclear\textsuperscript{13} although it has become first line therapy and suggested to have benefit for the majority of patients for almost half a century\textsuperscript{14}. Our data suggest however that the response rate may be more modest than currently generally considered.

This retrospective clinical and literature evaluation collates the single largest evaluation of genotyped hypoPP patients reported. Our observations indicate that acetazolamide has a benefit for at most only 50% of patients. However, we observed that the response rate improves to 60% if only those patients with common \textit{CACNA1S} mutations are considered. Furthermore, there is a suggestion that patients with arginine substitutions to glycine in the residues of the voltage sensor near the extracellular side of the sarcolemma may be predicted to respond poorly and this is supported by experimental observations. Conclusive evidence for a unified approach to the treatment of hypoPP is lacking and retrospective data as outlined here has limitations. However, the data reported here
support the view that randomised controlled trials of available therapies are required. Furthermore, given that the response rate is in the order of 50% we suggest new therapies need to be developed for patients with hypoPP.

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<th>Gene</th>
<th>Mutation</th>
<th>Total number of genotyped pts treated with ACZ</th>
<th>Beneficial effect</th>
<th>Deleterious effect</th>
<th>No response</th>
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**TABLE 1: SUMMARY OF RESPONSE TO ACETAZOLAMIDE BY GENOTYPE FOR COHORT 1 (UK CASES)**

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<th>Total number of genotyped pts treated with ACZ</th>
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<th>Deleterious effect</th>
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**TABLE 2: SUMMARY OF RESPONSE TO ACETAZOLAMIDE BY GENOTYPE FOR COHORT 3 (PUBLISHED CASES)**
Figure Legend

FIG 1: A: Diagrammatic representation of Cav1.1 with voltage sensor mutations known to cause hypoPP highlighted. B: Diagrammatic representation of Nav1.4 with voltage sensor mutations known to cause hypoPP highlighted. *R675G/Q/W has been associated with an atypical phenotype denoted potassium sensitive normokalaemic periodic paralysis. Figure modified with permission from The Journal of Physiology.13

Figure Title

Voltage sensor mutations of Cav1.1 and Nav1.4
Reference List


