Response to Mivacurium in a Patient Compound Heterozygous for a Novel and a Known Silent Mutation in the Butyrylcholinesterase Gene

Genotyping by Sequencing

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Background: Patients who are homozygous for the atypical mutation, compound heterozygous for atypical and silent mutations, or homozygous for silent mutations (SS) respond to mivacurium with extensively prolonged neuromuscular block. Although important, exact phenotyping of these patients is difficult. This article presents the pharmacodynamics and pharmacokinetics of a normal dose of mivacurium in a patient with genotype SS, including a pedigree analysis and delineation of the molecular genetic method used to identify the genotype.

Methods: The neuromuscular block following administration of mivacurium, at a dose of 0.14 mg/kg, was monitored in a 30-yr-old healthy man with use of a mechanosensor and mechanomyography, and times to different levels of recovery were measured. Venous samples for determination of the mivacurium isomers were collected during the interval 134–494 min after administration of mivacurium, and the terminal half-lives were calculated. Butyrylcholinesterase activity, phenotype, and genotype were determined for both the patient and the family. Complete nucleotide sequencing was used to identify the genotype.

Results: A train-of-four ratio of 0.75 was reached 469 min after the injection of mivacurium. The terminal elimination half-lives of the mivacurium isomers, cis-trans and trans-trans, were 90 min. Complete nucleotide sequencing revealed two point mutations, the known silent variant S7 and a previously undescribed mutation of amino acid residue 170 introducing a stop codon.

Conclusions: The patient was compound heterozygous for silent mutations in the butyrylcholinesterase gene. The response to mivacurium was an extensively prolonged duration of action. Identification of the rare silent mutations presupposes access to modern molecular genetic methods such as complete nucleotide sequencing.

IN patients with the usual butyrylcholinesterase gene (BCHE; plasma cholinesterase gene), mivacurium is rapidly hydrolyzed in plasma and the duration of action is short. However, about 24.0% of the white population carries at least one genetic variant allele of the butyrylcholinesterase enzyme (BCHE; E.C. 3.1.1.8), which may provide access to modern molecular genetic methods such as complete nucleotide sequencing.

Before the implementation of molecular genetic methods, blood samples from patients with suspected abnormal phenotypes were analyzed by means of a combination of conventional laboratory methods, such as measurement of BCHE activity and biochemical inhibitor reactions, and analyses of pedigrees. Unfortunately, the results of these investigations do not always allow correct phenotyping or correct prediction of the response to muscle relaxants, and as many as 25% of patients referred to the Danish Cholinesterase Research Unit cannot be classified by means of traditional biochemical methods. With techniques of DNA analysis it is possible to establish the genotype of a patient, and at least four articles have been published on clinical response in patients with genotypes AA and AS. However, none have documented the genotype of a patient with SS phenotype to whom mivacurium has been administered.

This article presents the pharmacodynamics and pharmacokinetics of a normal dose of mivacurium in a patient with genotype SS, along with a pedigree analysis and description of the molecular genetic method (complete nucleotide sequencing of BCHE) used to identify the genotype, i.e., compound heterozygous occurrence for two silent mutations.

This article is featured in “This Month in Anesthesiology.”

Please see this issue of ANESTHESIOLOGY, page 5A.
**Materials and Methods**

**Case Report**

The patient was a healthy 30-yr-old man (American Society of Anesthesiologists physical status I) scheduled for correction of the inner nose. He was 182 cm tall and weighed 73 kg. He reported no medications and had no known history of allergies. The patient had been anesthetized previously without any known complications, and low BChE activity was not suspected. Presurgical medication consisted of diazepam (10 mg) and acetaminophen (1 g orally). Anesthesia was induced with a continuous infusion of remifentanil (0.5 µg · kg⁻¹ · min⁻¹), and after 3 min of infusion, a bolus dose of propofol (145 mg). Mivacurium was given as a single dose of 10 mg (0.14 mg/kg) to facilitate tracheal intubation. Anesthesia was maintained with continuous infusions of remifentanil and propofol and 33% oxygen in air. Ventilation was adjusted to maintain normocapnia (end-tidal carbon dioxide, 35–42 mmHg). The central and peripheral skin temperatures were measured and kept above 36 and 32°C, respectively.¹⁴

**Neuromuscular Monitoring**

The neuromuscular block was monitored with a Datex Engstrom neuromuscular transmission module (MechanoSensor; Datex, Helsinki, Finland) with use of train-of-four (TOF) nerve stimulation. At completion of surgery, 90 min after the administration of mivacurium, no response to TOF nerve stimulation or tetanic stimulation (no posttetanic counts) could be detected. Sedation with propofol and ventilation were maintained, and the patient was taken to the intensive care unit. Here the mechanical twitch was recorded with a Myograph 2000 (Biometer International, Organon Teknika, Boxtel, The Netherlands). The ulnar nerve was stimulated at the wrist with surface electrodes and TOF nerve stimulation. The neuromuscular block was monitored continuously until a TOF ratio of 0.75 was obtained. End control was used as reference, and the following times were recorded: (1) time from start of injection of mivacurium to the first (T₁), second, and third responses to TOF stimulation; (2) times to different levels of first response in TOF (T₁) recovery; and (3) time to a TOF ratio of 0.75. The infusion of propofol was discontinued when the neuromuscular block had recovered to a TOF ratio of 0.75, and 5 min later the patient was awake and the trachea was extubated. At that time the patient was able to maintain head lift for 10 sec and cough sufficiently. He was observed in the intensive care unit for a further 3 h, after which he was discharged to the surgical ward. Recovery was uneventful and he was discharged to home the following day.

**Pharmacokinetic Methods**

Venous sampling was not possible until the patient had arrived in the intensive care unit. Twelve samples were collected in the interval from 134 to 494 min after the administration of mivacurium. In fewer than 10 s, the blood samples were transferred into a blood sample vial containing a cholinesterase inhibitor (phospholine iodide). The samples were centrifuged, and the plasma was decanted and frozen at −70°C. The ratios of the cis-cis, cis-trans, and trans-trans isomers in the clinical trial material used are approximately 5.8, 35.5, and 58.8%, respectively (data from Certificate of Analysis, Glaxo Wellcome, Beckenham, UK). The concentration of each isomer was determined by a stereospecific high-performance liquid chromatographic method with fluorometric detection and a stepped gradient, at the Faculty of Pharmacy, Montreal.¹⁵ The coefficient of variation was less than 8% and the lowest level of quantification was 5 ng/ml. Calibration was linear over the range of 5–5,000 ng/ml. The plasma concentrations of the cis-trans and the trans-trans isomers were used to construct a plasma concentration-versus-time profile of the drug. The terminal half-lives of these isomers of mivacurium were calculated from the equation

$$t_{1/2} = \frac{0.693}{\beta}.$$
and extension, amplification of exon 2A with primers 2F and 2.2R (fig. 1) was carried out with 2.5 units of AmpliTaq Gold (Perkin Elmer, Allerod, Denmark) instead of Taq polymerase. Amplified DNA samples showed single strong bands of the expected size, after agarose gel electrophoresis.

After purification of the PCR products (Jetquick Spin Column PCR Purification Kit, GENOMED; Ross-Petersen, Horsholm, Denmark), cycle sequencing was performed with dye-labeled dideoxynucleotides. To cover the entire BCHE region, 12 sequencing primers were used (table 1). The sequencing mixture contained 2.0–4.0 μl ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Mix (Applied Biosystems, Naerum, Denmark), 3.2 pmol primer, a variable amount of PCR product (depending on the sizes of the band visualized on the gel electrophoresis), and deionized water, adding up to 20 μl. The Terminator Ready Reaction Mix consists of the 4 dideoxynucleotides, each labeled with a dye terminator, AmpliTaq DNA polymerase, MgCl₂, and Tris-HCl buffer (pH, 9.0). Cycle sequencing was carried out on an Eppendorf Master Cycler Gradient Thermocycler, with use of 35 cycles at 96°C for 30 seconds, followed by 50°C for 15 seconds and, finally, 60°C for 4 minutes. To remove excess dye terminator, the extension products were purified by ethanol–sodium acetate precipitation.

The sequence reaction products were analyzed on an automatic ABI Prism 377 or 310 DNA sequencer (Applied Biosystems). The resulting sequences are presented as electropherograms.

The nucleotide sequence of the BCHE gene was determined, and mutations were detected by direct comparison with the sequence of the normal genotype. When mutations were detected, they were confirmed by sequencing in the opposite direction.

**Pedigree Analysis**

Retrospectively, the patient gave written informed consent to publish the results and permission to ask his family to participate in a pedigree analysis. After giving informed written consent, the parents and two brothers had blood samples drawn to determine BChE activity, phenotype, and genotype. The patients were a part of a study approved by the local ethics committee for Copenhagen, Denmark.

**Results**

**Pharmacodynamic Data**

Times to posttetanic counts of 1, 3, and 6 were 110, 157, and 189 min, respectively, and times to the first, second, and third response to TOF nerve stimulation were 274, 324, and 347 min, respectively. Spontaneous

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**Table 1. Primer Sequences Used to Amplify and Sequence the Butyrylcholinesterase Gene**

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Location</th>
<th>Primer Sequence (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>1F</td>
<td>5' End of gene (nt − 71 to nt − 49)</td>
<td>TTT CAA GTT GCT GCC GCC AAC TC</td>
</tr>
<tr>
<td>1R</td>
<td>5' End of intron 1 (nt 127 to nt 104)</td>
<td>GTA ACT GTT CCC CAC AGA GCC</td>
</tr>
<tr>
<td>2F</td>
<td>3' End of intron 1 (nt 130 to nt 96)</td>
<td>GAA CTA TAG GTT GGG TTC GCT AGG</td>
</tr>
<tr>
<td>2.2F</td>
<td>Exon 2 (nt 432 to nt 455)</td>
<td>TAG GAA TAT TGG CAC</td>
</tr>
<tr>
<td>2.2R</td>
<td>Exon 2 (nt 910 to nt 886)</td>
<td>GTT GCA TGT CAG TGG GAA AAT CAC C</td>
</tr>
<tr>
<td>2R</td>
<td>5' End of intron 2 (nt 188 to nt 164)</td>
<td>GAC ACA GGG AGT TGA AAT GCC GTT</td>
</tr>
<tr>
<td>3F</td>
<td>3' End intron 2 (nt 163 to nt 140)</td>
<td>CCA CTA AGC CCA GGT TCA GAT AGG</td>
</tr>
<tr>
<td>3R</td>
<td>5' End of intron 3 (nt 109 to nt 87)</td>
<td>GTC AGA GAT ACA TAT AGT AAC TTC</td>
</tr>
<tr>
<td>4F</td>
<td>3' End intron 3 (nt 100 to nt 74)</td>
<td>CTG TAG TGT GTA GTT AGA GAA AAT GCC</td>
</tr>
<tr>
<td>4R</td>
<td>3' End of gene (nt 524 to nt 499)</td>
<td>GGT CAT TTA AGG TGG CTA AAT CTC</td>
</tr>
<tr>
<td>2.1F*</td>
<td>Exon 2 (nt 77 to nt 101)</td>
<td>CAG GTT TTT GGA TGG GTG TAT</td>
</tr>
<tr>
<td>2.1R*</td>
<td>Exon 2 (nt 455 to nt 432)</td>
<td>CTT AGG GCC GCC ACC CTA TAG TTT</td>
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<td>2.3F*</td>
<td>Exon 2 (nt 871 to nt 895)</td>
<td>GGT CCC ACC GGT CAT GGT CAT TTT</td>
</tr>
<tr>
<td>2.3R*</td>
<td>Exon 2 (nt 1248 to nt 1221)</td>
<td>GGG ATT ATT CTC CGG TCC TGA GAA G</td>
</tr>
<tr>
<td>4.1R*</td>
<td>3' End of gene (nt 277 to nt 253)</td>
<td>GGA AAG AAA AAA ATT GAA CCA GGC GGC</td>
</tr>
</tbody>
</table>

Primers in introns are numbered according to the number of bases from the nearest intron–exon junction. Primers within the exons are numbered as indicated by Arpagaus et al.17

* Additional sequencing primers.

nt = nucleotide.

Fig. 1. Structural characteristics of the butyrylcholinesterase gene. The locations of the three introns and four exons are indicated. The location and direction of the amplification primers for exon 1 (1F and 1R), exon 2A (2F and 2.2R), exon 2B (2.2F and 2R), exon 3 (3F and 3R), and exon 4 (4F and 4R) are indicated by arrows. The white boxes indicate untranslated sequence; the black box is the leader sequence; and the dotted area is the coding region for the mature enzyme. UTR = untranslated region.
recovery to different levels of first response in TOF (T₁) is presented in figure 2. A TOF ratio of 0.75 was reached 469 min after the injection of mivacurium.

**Pharmacokinetic Data**

Figure 3 shows the plasma concentrations of the two active isomers over time. The terminal elimination half-lives of both isomers were 90 min. The plasma concentration of the cis-cis isomer was measurable in only the first sample; hence, estimation of the half-life of this isomer was not possible.

**Phenotypes**

The patient’s BChE activity was zero, indicating that he was homozygous for silent mutations. A pedigree and the biochemical data for the patient and his family are shown in figure 4. The biochemical data indicate that the patient and one brother are homozygous for silent mutations, whereas the father, the mother, and the other brother are heterozygous for a silent mutation.

**Molecular Genetic Profile**

The DNA analysis revealed two point mutations at exon 2. One was the known silent variant S7, a point mutation (G→A) at nucleotide 344, changing amino acid residue 115 from glycine to aspartate (Gly115Asp) (fig. 4). The other mutation, which has not previously been described, introduced a stop codon (CAG to TAG) at nucleotide 514, changing the amino acid residue 172 from glutamine to termination of the sequence (Gln172STOP) (fig. 4). Thus, the patient is compound heterozygous for two silent mutations. Accordingly, the parents are both heterozygous for one silent mutation. One brother has the same genotype as the patient, whereas another brother is heterozygous for the S7 mutation (fig. 4). The formal names of S7 and the new variant are BCHE*115D and BCHE*172STOP, respectively, according to the proposed nomenclature for BChE variants.

**Discussion**

The case presented shows that patients compound heterozygous for two silent mutations respond to mivacurium with extensively prolonged duration of action. The time to sufficient clinical recovery of neuromuscular function was 8 h after a normal intubation dosing. In addition, the case illustrates that correct genotyping of the patient and his family, which was not possible with traditional biochemical methods, necessitated a molecular genetic approach including complete nucleotide sequencing of the coding region of BChE. Thus, the presence of a previously undescribed mutation was disclosed.

**Pharmacodynamics**

Patients with phenotypes SS, AS, or AA all have no or very little active BChE. A prolonged duration of action of mivacurium is therefore to be expected in these patients. The extremely prolonged duration of action of mivacurium in our patient, who was compound heterozygous for two silent mutations, correlates well with the findings described in other reports. In a patient homozygous for the atypical gene, Maddineni and Mira-Khur found that the time to first response to nerve stimulation, measured by mechanomyography, was 4 h. In other cases, complete recovery from the neuromuscular block documented with mechanomyography or acceleromyography took 7 or 8 h. Finally, in some investigations in which objective monitoring methods were not used, complete or partial neuromuscular blocks have been found to last 3–8 h.

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*Fig. 2. Spontaneous recovery after administration of mivacurium at a dose of 0.14 mg/kg to a patient who was compound heterozygous of two silent mutations (open squares). Mean recovery profile after administration of mivacurium (0.2 mg/kg) to 20 phenotypically normal patients (open circles) is shown for comparison. T₁ is the first response to train-of-four nerve stimulation.*

*Fig. 3. The logarithm of the plasma concentrations (ng/ml) of the cis-trans isomer (open triangles) and the trans-trans isomer (closed triangles) of mivacurium over time in a patient who was compound heterozygous of two silent mutations.*
The ED\textsubscript{95} has been estimated at 16 µg/kg in a patient with phenotype SS and 20 µg/kg in patients homozygous for the atypical mutation AA.\textsuperscript{20} Accordingly, 1.5–2 times the ED\textsubscript{95} (30 µg/kg) caused 100% neuromuscular block and a time to reappearance of T\textsubscript{1} of approximately 55 min (30–130 min) in five patients with AA phenotype.\textsuperscript{2} Therefore, it is not surprising that nearly five times this dose (mivacurium, 140 µg/kg) administered to our patient caused a very prolonged block, with reappearance of T\textsubscript{1} after 274 min.

**Pharmacokinetics**

The concentrations of the cis-trans and the trans-trans isomers were 45 and 65 ng/ml, respectively, in the first sample, obtained 134 min after the administration of mivacurium. This is in agreement with data for patients homozygous for the atypical mutation (\textit{Östergaard et al.: ANESTHESIOLOGY} 1997; 87(suppl 3A):A854 [abstract]).

The active isomers were present in plasma up to 419 min after the administration of mivacurium. This is much longer than in normal patients or in patients with decreased BChE activity due to organ failure. In healthy patients, the cis-trans and the trans-trans isomers were measurable for 20 and 30 min, respectively.\textsuperscript{21} In patients with normal phenotypes but decreased BChE activity, the corresponding values were 30 and 40 min, respectively.\textsuperscript{21} Furthermore, in patients with bambuterol-induced low BChE, the isomers were detectable for 60 min.\textsuperscript{22} Data from a study of patients with renal failure indicated that the cis-cis isomer was partly excreted through the kidney and that the rate of clearance of this isomer was less affected by low BChE activity than that of the active isomers.\textsuperscript{23} This might explain why the cis-cis isomer concentration in plasma was low in our patient when sampling started. Most probably, the cis-cis isomer is eliminated by several pathways,\textsuperscript{23} of which hydrolysis by BChE and renal elimination represent only two.

The terminal elimination half-lives of the cis-trans and trans-trans isomers were about 90 min in our patient. This is extremely prolonged in comparison with the half-lives of these isomers in healthy adults (1.5 and
In phenotypically normal patients, mivacurium is rapidly hydrolyzed by BChE and the determinant of drug effect is thus drug elimination rather than distribution. In our patient, however, the determinant was drug distribution, as the patient has no active BChE. Although the plasma concentration decreased and the volume of distribution increased with time, the drug concentration at the receptor site remained high, and recovery from the neuromuscular block was therefore slow.

The pharmacokinetic results might be affected by the fact that venous sampling was not started until 134 min after mivacurium was administered. As early sampling was not possible, we cannot calculate the area under the curve and hence the clearance. The importance of early sampling is minor, however, for a drug with an elimination half-life of 90 min. In this case, the length of sampling period is important. We do not know the maximum concentrations of the different isomers of mivacurium achieved in this patient, but the concentrations are most likely comparable to the maximum concentrations in patients homozygous of the atypical mutation A.

**DNA Analyses of the Kindred**

DNA analysis revealed two point mutations, the known S7-variant and an undescribed mutation, which introduced a stop codon at amino acid residue 172 of the 574 amino acid residues of normal BChE. Biochemical data supported the conclusion that the patient was compound heterozygous for two silent mutations. Pedigree analyses showed that S7 was inherited from the mother and the novel mutation from the father, which proves that both alleles are affected in the patient (fig. 4).

The pharmacodynamics of mivacurium in patients homozygous for the atypical mutation and compound heterozygous for a silent and the atypical mutation have been reported. However, there are no previous reports of pharmacodynamic or pharmacokinetic data of mivacurium in patients carrying two genotypically determined silent mutations.

The homozygous silent phenotype of BChE is characterized by complete absence of BChE activity or by less than 10% activity of the usual phenotype. Heterogeneity of this phenotype is well established, and at DNA level 30 silent variants are known. Heterozygous occurrence of the silent mutation is estimated to be 1:200 patients, whereas homozygous silent and compound heterozygous silent mutations are very rare (1:30,000–1:100,000). Complete nucleotide sequencing of the coding region of BChE is necessary, however, for identification of the silent mutations that are located throughout the coding region.

Screening for all abnormal BChE genotypes is not possible in daily clinical practice, as such analysis is time-consuming and expensive. It should be used to supplement equivocal results obtained by biochemical methods, however. Faster and inexpensive genotyping methods for determination of the most common mutations, e.g., the A and the K variants, are desirable. In the future, the Danish Cholinesterase Research Unit will apply complete analysis of the genotype in cases in which it is clinically important to establish the genotype.

In conclusion, our results indicate that patients who are compound heterozygous for silent mutations in the BChE respond to mivacurium by extensively prolonged duration of its action, and identification of the rare silent mutations presupposes access to modern molecular genetic methods, such as complete nucleotide sequencing.

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**References**

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