Response to Mivacurium in Patients Carrying the K Variant in the Butyrylcholinesterase Gene

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Background: Mivacurium is hydrolyzed by the butyrylcholinesterase enzyme, and patients with hereditary changes of the enzyme often have prolonged duration of action of mivacurium. In this study, the authors investigated the significance of the most commonly occurring variant, the Kalow (K) variant, established using DNA analysis, for the response to mivacurium.

Methods: A total of 58 patients carrying either the wild-type butyrylcholinesterase or different combinations of the atypical (A) variant and the K variant were included. Patients who were homozygous for the A variant were given 0.03 mg/kg mivacurium. All other patients received 0.2 mg/kg mivacurium. The neuromuscular block was measured using train-of-four nerve stimulation and mechanomyography. Genotyping was performed with complete nucleotide sequencing.

Results: Heterozygosity of the K variant prolonged the time to train-of-four 0.70 from 26.6 to 34.5 min (30%; not significant) as compared with the wild type. Heterozygosity of the K variant linked to the A variant prolonged the corresponding time from 32 to 42.7 min (33%; P = 0.03) as compared with patients who were heterozygous for solely an A allele. For eight patients who were homozygous for both the A and K variants, the time to 25% recovery was 78–89 min as compared with 44–57 min in patients who were homozygous for the A variant or had only one linked K variant.

Conclusion: The K variant prolongs the duration of action of mivacurium. The current results indicate that the effect is modest when the K variant occurs heterozygously with the wild type or the A variant but is marked in patients who are homozygous for both the A and K variants.

MIVACURIUM is rapidly hydrolyzed by the butyrylcholinesterase enzyme (BChE; plasma cholinesterase). However, approximately 25% of individuals in a white population have a hereditary abnormal enzyme,1 and this may result in slow hydrolysis of mivacurium and a prolonged neuromuscular block.2 The butyrylcholinesterase gene (BCHE) is located on chromosome 3 (3q26.1–q26.2).3 More than 40 genetic variants of BCHE have been described,3 of which the most common variants are the atypical (A) variant and the Kalow (K) variant, with allelic frequencies of 0.02 and 0.128–0.21, respectively.3–7

The A variant is the result of a point mutation (nt 209 GAT→GGT), which is manifested by an amino acid change Asp70→Gly. Patients who are homozygous for the A variant are very sensitive to mivacurium, and extensively prolonged duration of action has been reported. Therefore, after a usual dose of 0.12–0.2 mg/kg mivacurium, the time to full spontaneous recovery of neuromuscular function is 6–8 h,8–11 as opposed to approximately 30 min in patients with normal BChE.12

The K variant is caused by the point mutation (nt 1615 GCA→ACA) resulting in the amino acid change Ala539→Thr. The significance of this variant for the duration of action of mivacurium is unknown.

There is a linkage between the K variant and the A variant, and the K variant is present in 89% of BChE genes containing the A variant.5 The clinical significance of this linkage has not been determined.

Identification of the K variant is difficult and often impossible using biochemical assays on BChE activity and biochemical inhibitor reactions. Therefore, when using only biochemical assays, many patients carrying the K variant are classified as phenotypically heterozygous or homozygous for the A variant only.5 Correct genotyping that identifies all variants requires detailed DNA analysis.

The purpose of this study was to investigate the significance of the K variant for the response to mivacurium in patients in whom the genotypes were established using DNA analysis.

Materials and Methods

A total of 58 adult patients in whom the genotype was established using DNA analysis were included in the study. The patients had previously been issued with warning cards by the Danish Cholinesterase Research Unit, requesting them and the anesthesiologist to contact the Research Unit if they were to undergo surgery. The investigator from Danish Cholinesterase Research Unit (an anesthesiologist) traveled with the necessary equipment to the hospital where surgery was to take place. The regional ethics committee (Copenhagen, Denmark), approved the study, and written informed consent was obtained from the patients. We excluded pregnant women and patients with a history of neuromuscular, cardiovascular, renal, or hepatic disorders, as well as patients receiving drugs that might affect neuro-
muscular transmission. In all patients, the following variables were recorded: age, weight, height, diseases, and drugs that might influence BChE activity.

**Determination of Genotype**

Butyrylcholinesterase activity was measured using the method of Kalow and Lindsay, with the use of benzoylcholine as the substrate. Genotyping was performed using complete nucleotide sequencing. In brief, genomic DNA was extracted from leukocytes. The four exons and intron-exon boundaries of the BCHE gene were amplified in five polymerase chain reactions. The products of the polymerase chain reactions were purified, and after cycle sequencing with dye terminators, the products were analyzed on an automatic ABI Prism DNA 377 sequencer (Applied Biosystems, Copenhagen, Denmark). In this way, the nucleotide sequence of the BCHE gene was determined, and mutations were detected by direct comparison with the sequence of the normal genotype. The method of sequencing double-stranded DNA does not distinguish between mutations on different alleles and those on the same strand. Therefore, pedigree analysis was also performed whenever possible.

The nomenclature of the BCHE genetic variants is established to allow separate description of the two alleles: One allele contains two mutations, the A and the K variants, whereas the second allele carries only the K variant.

**Anesthesia**

The period of enrollment of patients in different clinical investigations lasted 10 yr. Therefore, the type of anesthesia varied slightly over the years. All patients received intravenous anesthesia, with or without 66% nitrous oxide in oxygen. Ventilation was controlled, the aim being to keep the patient normocapnic using capnography (end-tidal carbon dioxide 34–42 mmHg). In the earlier studies, anesthesia was induced with thiopental, droperidol, and fentanyl, and supplementary doses of fentanyl were given as needed. In recent studies, induction with opioid/propofol was followed by a continuous infusion of propofol, and opioid boluses were given when required.

**Doses of Mivacurium**

The dose of mivacurium varied depending on the genotype in question. Patients who were homozygous for the A variant, whether linked with the K variant or not (A/A, AK/A, and AK/AK), were given 0.03 mg/kg intravenous mivacurium. Patients carrying the wild type (U/U) and patients with heterozygous occurrence of the A variant or with heterozygous or homozygous occurrence of the K variant (U/K, K/K, U/A, U/AK, and K/AK) received 0.2 mg/kg intravenous mivacurium. Mivacurium was given over 20 s.

**Neuromuscular Monitoring of the Response to Nerve Stimulation**

Neuromuscular monitoring was performed in accordance with Good Clinical Research Practice in pharmacodynamic studies of neuromuscular blocking agents. After induction of anesthesia, the ulnar nerve was stimulated supramaximally every 12 s using train-of-four (TOF) nerve stimulation. The evoked response from the adductor pollicis muscle was measured using mechanomyography (Myograph 2000; Biometer, Odense, Denmark). The palmar skin temperature was kept above 32°C, and the central temperature was kept above 35°C. After supramaximal stimulation was achieved and the response to stimulation was stable for 2–3 min, the control response of the first response in TOF (T1) = 100% was measured, and mivacurium was given. Recovery data were compared using start control value. Whenever possible, the twitch response was allowed to recover spontaneously at the end of surgery. If necessary, residual block was antagonized with neostigmine. Monitoring was continued until at least 90% recovery twitch height or a TOF ratio of 0.70–0.80. Sustained head lift for 5 s was ensured.

In two cases, technical problems with the myograph occurred before injection of mivacurium. As an alternative, the acceleromyography-based TOF-Guard (Organon Teknika, Boxtel, The Netherlands) was used. In these cases, the acceleration transducer of the TOF-Guard was fixed over the distal interphalangeal joint of the thumb. As with mechanomyography, a stable response for 3 min was ensured before the control response was defined.

The duration of neuromuscular block was defined as the time from the start of injection to the first response to TOF stimulation T1, to 10% T1 twitch recovery (duration T1 10%), to 25% T1 recovery (duration T1 25%), and to 75% and 90% T1 recovery. Also, the time to TOF 0.70 was recorded.

The primary endpoints were the times to the first response to TOF (T1) and to TOF 0.70.

**Statistics**

Data are reported as median and range. The Mann-Whitney test was used for comparing duration of action of mivacurium in the different genotypes (U/U vs. U/K and U/A vs. U/AK). P < 0.05 was considered statistically significant.

**Results**

**Patient Characteristics**

Of the 58 patients, 25 were men and 33 were women. They ranged in age from 19–75 yr (weight, 42.5–110 kg;
Genotype alleles are separated by a slash.

BCHE = butyrylcholinesterase; T1 = time to reappearance of the first response to train-of-four; T1 10% = 10% recovery of the first response to train-of-four; T1 25% = 25% recovery of the first response to train-of-four.

Table 1. BChE Activity and Individual Recovery Data after a Low Dose of Mivacurium (0.03 mg/kg) in Patients with Different BCHE Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype n</th>
<th>BChE Activity, U/l</th>
<th>T1</th>
<th>T1 10%</th>
<th>T1 25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>17</td>
<td>1,115 (604–1,591)</td>
<td>13.8 (7.4–18.3) (n = 16)</td>
<td>15.0 (9.3–21.7)</td>
<td>17.6 (10–25.8)</td>
</tr>
<tr>
<td>AK/A</td>
<td>9</td>
<td>591 (275–946)</td>
<td>19.8 (11.4–29.0)</td>
<td>20.5 (12.6–33.4)</td>
<td>23.4 (14.0–29.4)</td>
</tr>
<tr>
<td>AK/AK</td>
<td>4</td>
<td>550 (433–785)</td>
<td>21.1 (16.8–23.6)</td>
<td>23.3 (19.8–27.8)</td>
<td>27.1 (21.9–32.6)</td>
</tr>
<tr>
<td>U/A</td>
<td>2</td>
<td>815 (703–927)</td>
<td>16.5 (14.3–18.5)</td>
<td>19.7 (17.3–22.0)</td>
<td>22.3 (19.8–24.8)</td>
</tr>
<tr>
<td>U/K</td>
<td>11</td>
<td>742 (479–986)</td>
<td>24.8 (19.0–35.0)</td>
<td>27.4 (21.3–41.0)</td>
<td>31.5 (26.0–44.0)</td>
</tr>
<tr>
<td>U/AK</td>
<td>5</td>
<td>699 (583–936)</td>
<td>22.1 (19.0–29.0)</td>
<td>24.0 (22.0–31.6)</td>
<td>28.9 (24.0–35.6)</td>
</tr>
<tr>
<td>AK/AK</td>
<td>2</td>
<td>578 (577–579)</td>
<td>31.9 (30.5–33.3)</td>
<td>33.0 (32.7–33.3)</td>
<td>39.6 (37.7–41.5)</td>
</tr>
</tbody>
</table>

Duration, min

Table 2. BChE Activity and Recovery Data after a Normal Dose of Mivacurium (0.2 mg/kg) in Patients with Different BCHE Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype n</th>
<th>BChE Activity, U/l</th>
<th>T1</th>
<th>T1 10%</th>
<th>T1 25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>U/U</td>
<td>17</td>
<td>1,115 (604–1,591)</td>
<td>13.8 (7.4–18.3) (n = 16)</td>
<td>15.0 (9.3–21.7)</td>
<td>17.6 (10–25.8)</td>
</tr>
<tr>
<td>U/K</td>
<td>9</td>
<td>591 (275–946)</td>
<td>19.8 (11.4–29.0)</td>
<td>20.5 (12.6–33.4)</td>
<td>23.4 (14.0–29.4)</td>
</tr>
<tr>
<td>U/A</td>
<td>2</td>
<td>815 (703–927)</td>
<td>16.5 (14.3–18.5)</td>
<td>19.7 (17.3–22.0)</td>
<td>22.3 (19.8–24.8)</td>
</tr>
<tr>
<td>U/K</td>
<td>11</td>
<td>742 (479–986)</td>
<td>24.8 (19.0–35.0)</td>
<td>27.4 (21.3–41.0)</td>
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</tr>
<tr>
<td>AK/AK</td>
<td>2</td>
<td>578 (577–579)</td>
<td>31.9 (30.5–33.3)</td>
<td>33.0 (32.7–33.3)</td>
<td>39.6 (37.7–41.5)</td>
</tr>
</tbody>
</table>

Data are presented as median (range). Genotype alleles are separated by a slash. When linkage between variants is not established, they are shown in brackets.

n = number of patients; T1 = time to reappearance of the first response to train-of-four; T1 10% = 10% recovery of the first response to train-of-four; T1 25% = 25% recovery of the first response to train-of-four; TOF = train-of-four.

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Patients with Wild Type, Homozygous for the K Variant or Heterozygous for the A Variant, Combined with the Wild Type or the K Variant (n = 50)

Table 2 shows the recovery data and BChE activity for the different genotypes. In 11 patients with an A and a K variant, an allelic separation of the mutations was not established because no pedigree analysis could be performed (patients in brackets, table 2). However, most probably these 11 patients are heterozygous for AK (U/AK). First, Bartels et al.5 found the K variant in 89% of BCHE genes containing A variant linkage. Second, all 11 patients were phenotyped using the biochemical inhibitor reactions Dibucaine number 18 and Ro number 19, indicating that they were U/AK and not A/K.20 Data from these 11 patients were therefore joined with data from the 5 patients who were certified as UAK and compared with data from the 2 patients with genotype U/A.

The presence of a K variant (U/K) prolonged the times to first response to TOF stimulation and to TOF = 0.70 from 13.8 to 19.8 min (43%; P = 0.01, Mann-Whitney test) and from 26.6 to 34.5 min (30%; not significant, Mann-Whitney test), respectively, as compared with the wild type (U/U).

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test) and from 32 to 42.7 min (33%; $P = 0.03$, Mann–Whitney test), as compared with patients who were heterozygous for an unlinked $A$ allele ($U/A$).

Relation between $BChE$ Activity and Duration of Action of Mivacurium

Figure 1 illustrates the relation between $BChE$ activity and time to first response to $T_1$ after 0.2 mg/kg mivacurium in patients with genotypes $U/U$, $U/K$, $K/K$, and $U/AK$.

Discussion

The main finding of this study is that the $K$ allele prolongs the duration of action of mivacurium. This effect is modest, but important because the $K$ variant is common and often coupled to the $A$ variant. From a biochemical point of view, an effect on duration of action of mivacurium is not surprising, although not described before. The $K$ variant is a quantitative variant that reduces $BChE$ activity by approximately 30%.21 Therefore, small effects of the $K$ variant alone and possibly also effects on other variants in cases of coupling of mutations might be expected.

Methods

This study is the first to evaluate the response to mivacurium prospectively and objectively in accordance with the Guidelines for Good Clinical Research Practice for studies in neuromuscular blocking agents16 in patients documented by molecular genetic methods to carry different combinations of $A$ and $K$ alleles in the $BChE$ gene.

It may be considered a weakness of the study that a TOF ratio of 0.70 was chosen as the final endpoint of recovery. However, at the time when the study was initiated, this ratio was considered to represent sufficient neuromuscular recovery. It has been documented that neuromuscular recovery should ideally be followed until a TOF ratio of 0.80 or even 0.90.16,22,23

It may also be considered a weakness that in the group of patients with abnormal genotypes, we decided to include all patients, irrespective of whether they received drugs or had a disease that might influence $BChE$ activity. The reason for this decision is that the application of the exclusion criteria would seriously limit the eligible number of patients. However, the patients taking medication were equally distributed on both sides of the medians in patients carrying $U/K$ or $U/AK$ and therefore most probably did not affect our results. The effect of estrogens on the duration of action of mivacurium is not known, but the $BChE$ activity is reduced up to 20% in patients receiving oral contraceptives.24 For calcium blockers, experimental studies have shown significant alterations in effects of neuromuscular blocking agents, but a clinical study showed no significant differences in the recovery time after rocuronium in patients receiving chronic therapy with a calcium blocker.25 The effect of calcium blockers on mivacurium has not been assessed, but the effect is probably minimal.

We performed genotyping using complete nucleotide sequencing of the four exons, the intron–exon boundaries of the $BChE$ gene, and in the untranslated region of the messenger RNA of our patients, because mutations other than the $A$ and $K$ variants should be excluded. Cerf et al.26 performed molecular analysis for specific detection of the $A$ variant only and may have missed other mutations, which could explain the prolongation of the neuromuscular block in their patients. It may explain the severely prolonged duration of paralysis (up to 600 min).
that they found in patients who were heterozygous for the A variant. In our study, patients who were heterozygous for the A variant experienced a duration of action of mivacurium of 35 min at the most.

In 11 patients, we were only able to make probable that their genotype was U/AK and not A/K. Pedigree analysis is necessary to document the genotype, but it was not possible in these cases. However, it does not change the fact that these patients have a longer duration of action of mivacurium than patients with genotype U/A only.

**Patients Homozygous for the A Variant, with or without Presence of the K Variant**

Although the number of patients is too small to allow for statistical evaluation, the data indicate that patients with genotypes A/A or AK/A have shorter durations of action of mivacurium than patients with the AK/AK genotype.

There are several recent case reports of extremely prolonged duration of action of mivacurium in patients with phenotype AA, but only two prospective controlled clinical trials evaluating the response to mivacurium objectively. In these studies, a large variation in response to mivacurium was found, most probably because of variation in the underlying genotypes. Only one patient with phenotype AA and an established genotype (A/AK) has had the response to mivacurium recorded objectively. Vanlinhout et al. found an extremely prolonged duration of action after a small dose of mivacurium, 0.015 mg/kg, in a patient who was compound heterozygous for the A and AK variants (A/AK). The duration $T_1$ 10% was 290 min, as compared with 32, 40, and 88 min in our three patients with the same genotype who were given 0.03 mg/kg mivacurium. The duration $T_1$ 10% found by Vanlinhout et al. is close to the duration $T_1$ 10% of 344 min found by us in a patient who was compound heterozygous for two silent mutations and who was given 0.14 mg/kg mivacurium, i.e., 10 times the dose given by Vanlinhout et al. We have no obvious explanation for this difference, but we do wonder whether the patient of Vanlinhout et al. might by mistake have received a higher dose of mivacurium than the stated 0.015 mg/kg.

Three patients who were later found by DNA analysis to be homozygous for the atypical allele have received 0.2 mg/kg mivacurium as part of a routine anesthesia. Postoperatively, the time to sufficient respiration was 6–8 h. However, apparently none of the patients were checked for the K allele, and the response to mivacurium was not recorded objectively.

**Patients with Wild Type, Homozygous for the K Variant or Heterozygous for the A Variant, with Combinations of K and U Variants**

Only one prospective controlled clinical trial evaluated the response to 0.2 mg/kg mivacurium in patients who were phenotypically heterozygous for the A or the K allele or both. However, in that study, the genotypes were not determined. Barta et al. and Cerf et al. reported two patients who were given 0.2 mg/kg mivacurium and showed insufficient respiration postoperatively for 5.5 and 4 h. Phenotypically, the two patients were heterozygous for the A variant, and molecular genetic analyses resulted in genotypes AK/K and U/A, but Cerf et al. did not genotype the K variant. The apparently long duration of postoperative respiratory insufficiency found in both patients is at variance with our findings. We have never recorded a time to TOF 0.70 of more than 70 min in any patient with phenotype UA or genotypes U/A, U/K, K/K, U/AK, or K/AK (table 2). The most obvious explanation is that the response to mivacurium was not recorded objectively in the patients of Barta et al. and Cerf et al., making the evaluation of the postoperative neuromuscular function difficult. Furthermore, Cerf et al. only genotyped the A variant, not the rest of the BCHE gene. Therefore, their patient may also have a silent variant and possibly the K variant.

**Clinical Significance of Cholinesterase Activity**

There is a major overlap in enzyme activity in the genotypes U/U, U/K, K/K, and U/A (fig. 1), and the relation between measured enzyme activity using benzoylcholine as substrate and the response to mivacurium is complex. Only for genotype U/U (and possibly U/K) do the results indicate a relation: The lower the enzyme activity is, the longer the effect of mivacurium is, an effect that has been reported previously. Figure 1 illustrates that it is not possible to predict a patient’s response to mivacurium solely on the basis of enzyme activity measured using benzoylcholine as the substrate.

**Clinical Significance of the K Variant**

The K variant is by far the most common clinically significant BCHE variant. Therefore, whenever mivacurium is given, there is a considerable risk that the patient has a K variant that will prolong the duration of action of mivacurium. If, for example, the patient is among the 25% who has genotype U/K, the duration of action will most probably be prolonged 30–40% (6–8 min) after a normal dose of mivacurium. We think this may be clinically relevant, depending on the circumstances. Because of its short duration of action, mivacurium is often used in short-term surgery when intense neuromuscular block is required for a short period of time, i.e., direct laryngoscopy. Under these circumstances, an unexpected although moderate prolongation of the block may matter. If objective neuromuscular monitoring is not applied in the daily clinical practice, the risk for residual neuromuscular blockade is further increased as the variability of the duration of action of mivacurium is large because of hereditary circumstances. The presence of one or two K variants linked to homozygous A vari-
nants will cause a neuromuscular block that will last many hours and significantly influence the work in the operating room and in the recovery ward.

Conclusion

Our data indicate that the K variant causes a prolongation of the action of mivacurium. The prolonged response is seen both when the K variant occurs heterozygously with the usual allele (U/K) or the atypical variant (U/AK), although it is most pronounced in patients who are homozygous for both the A and K variants.

References