

RELATIONSHIP BETWEEN SUCCINYLSCHOLINE-INDUCED APNEA AND DIBUCAINE NUMBER IN PATIENTS WITH GENETIC DEFICIENCY OF PLASMA CHOLINESTERASE

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ABSTRACT

In this study, a commercially available kit in which propionylthiocholine is used as a substrate and a dibucaine solution to determine the variants of the enzyme were utilized.

Plasma cholinesterase activity of 151 patients who underwent different operations was measured during a period of six months. The mean \pm SD of the enzyme activity in 150 patients was 4.39 \pm 1.17 kU/L.

Seventeen patients (11.2%) were found to have a serum cholinesterase activity of less than normal (<3 kU/L), but the period of apnea and the dibucaine number (DN) were found to be normal. Eight patients (5.3%) displayed an increased sensitivity to succinylcholine.

Six of these patients (4%) were heterozygous for atypical enzyme with a mean period of apnea of 18.6 min (range 12-35 minutes) and had a dibucaine number of less than normal (<80%). The DN of the other two patients (1.3%) who were homozygotes and had an exceedingly low atypical enzyme allele was 10% and 24%. The mean \pm SD DN value, in the 149 out of 151 patients was 84.41 \pm 2.91%, and the DN ranged from 10-90% in these patients. The mean \pm SD apnea period in the 149 patients was 6.37 \pm 4.28 min.

Keywords: Plasmacholinesterase, Succinylcholine, Apnea, Dibucaine number (DN), Propionylthiocholine, Homozygote

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INTRODUCTION

Suxamethonium has been widely used in anesthesia and surgical operations for more than four decades.

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Even today in modern anesthesia procedures, despite the fact that it is associated with a high incidence of complications, some patients demonstrate a prolonged response or period of apnea when the usual dosage is utilized. Thus it is imperative to determine plasma cholinesterase activity and the genetic predisposition of patients in the population.¹⁻⁴

Apnea and Dibucaine Number in Plasma Cholinesterase Deficiency

There are two types of plasma cholinesterase enzymes. True cholinesterase or acetylcholinesterase which is found in nervous tissue and the erythrocytic membrane, and atypical plasma cholinesterase or serum cholinesterase, pseudocholinesterase and butyrylcholinesterase (EC 3.1.1.8 acylcholine acylhydrolase) which is synthesized in the liver and which plays an important role in the breakdown and hydrolysis of suxamethonium.^{3,6}

The activity of plasma cholinesterase depends on acquired and genetic factors. A decreased activity of this enzyme could result in prolonged paralysis and apnea following administration of suxamethonium for endotracheal intubation.^{2,4,7}

The synthesis of the enzyme is controlled by an autosomal gene with different alleles. The "atypical" activity of plasma cholinesterase is diminished in patients who are homozygotes for one of these alleles and as such these persons exhibit an enhanced sensitivity to suxamethonium.

The major aim of the present study was to determine the sensitivity of patients to succinylcholine who may demonstrate a prolonged period of apnea and further determine whether they are homozygotes or heterozygotes, by measuring the plasma cholinesterase activity and also the dibucaine number (DN).

MATERIALS AND METHODS

Patients

For this study, 151 patients comprised of 92 males and 59 females operated upon in hospitals affiliated to Tehran and Iran Medical Universities in Tehran during a period of 6 months were studied without taking into consideration the gender or age of the patients.

Apart from this number, another three established cases of genetic enzyme deficiency were referred by colleagues. Blood samples were collected in these three cases as well, before the administration of suxamethonium.

Preparation of plasma samples

We collected 10 mL blood samples with heparin anticoagulant, centrifuged them (1000×g, 10 min), and separated and stored the plasma at -20°C until required.

Determination of the period of apnea

Before the induction of anesthesia, atropine 0.07 mg per kg body weight and sodium thiopental 5-7mg per kg body weight were administered intravenously to all the cases. Then suxamethonium 1.5 mg per kg body weight was given as a bolus intravenously to facilitate endotracheal intubation. The period of apnea was calculated from the time suxamethonium was administered until the time the patients regained spontaneous respiration.

Table I: Relationship between PChE and factors pertaining to apnea (APN) and dibucaine number (DN).

	PChE	DN	APN
PChE	1.0000	+ .4699**	-.3530*
APN	-.3530*	-.9383**	1.0000
N= 151			

(*): Between the duration of APN, PChE level and DN, APN a negative relationship exists.

(**): Between DN and PChE level, a positive relationship exists.

Table II: The duration of apnea (APN) and dibucaine number (DN) in relationship to the decrease in the activity of PChE.

PChE	DN(%)	APN(min)
2.52	87	4
1.93	85	4
2.37	85	6
2.22	84	4
2.67	84	6
2.67	83	3
2.97	83	5
2.97	83	6
2.52	82	6
2.52	79	14
3	79	35
2.82	78	18
1.93	76	12
2.07	75	17
1.93	70	16
1.93*	24	240

(*): Patients who were excluded from the study.

Investigation technique

In this study, a colorimetric method with propionylthiocholine as substrate was utilized. Plasma cholinesterase hydrolyzes propionylcholine to thiocholine, which reacts with dithiobisnitrobenzoate (DTNB) and produces 5-thio 2-nitrobenzoate anion which can be monitored by measuring its absorbance at 405 nm.^{4,8}

DN determination for abnormal pseudocholinesterase

As dibucaine inhibitor causes inhibition of the enzyme by linkage with plasma cholinesterase's active receptor, the enzyme is therefore rendered unable to hydrolyze propionylthiocholine substrate. Inhibition of cholinesterase activity by dibucaine is called the dibucaine number (DN), which is calculated by the following formula:

$$100 - \left[\frac{\text{PChE} - \text{D}}{\text{PChE}} \times 100 \right] = \text{inhibition percentage of cholinesterase activity by dibucaine.}$$

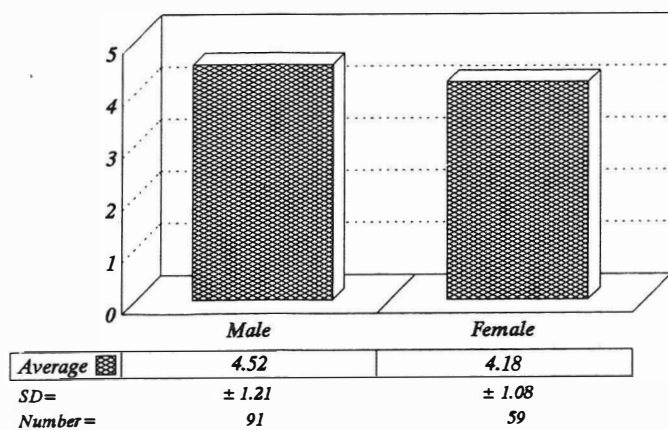


Fig. 1. Mean±SD activity of the enzyme (kU/L) in both sexes.

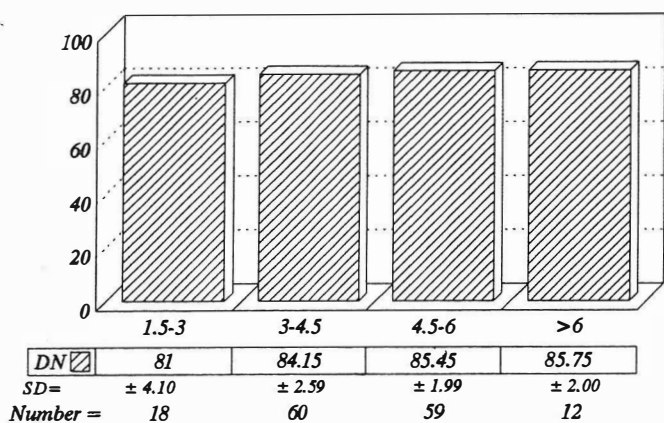


Fig. 2. Mean±SD DN(%) related to various enzyme activity (kU/L)

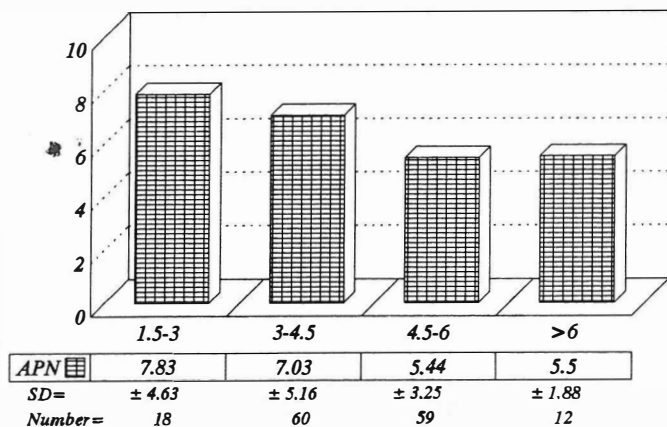


Fig. 3. Mean±SD apnea period (min.) and the related enzyme activities (kU/L).

where PChE= total plasma cholinesterase activity and PChE-D= cholinesterase activity in the presence of 0.3 mmol/L dibucaine solution.

Statistical analysis

The data were accumulated and with the help of a computer, the mean±SD and correlation and frequency

which are descriptive have been critically analyzed. The relationship between the level of plasma cholinesterase and duration of apnea following succinylcholine administration have been studied utilizing Pearson's correlation coefficient, regression equations and Student's t-test.

The unit of cholinesterase activity (kU/L) is expressed as millimoles of substrate hydrolyzed per minute per liter of plasma at 30°C.

RESULTS

Activity of plasma cholinesterase

The range of the plasma enzyme activity varied from 1.93-7.72 kU/L. The mean activity of the plasma enzyme in 150 patients was 4.39 kU/L and the standard deviation came as 1.17 kU/L. However one patient who was excluded from the study had an enzyme activity of 0.14 kU/L.

Out of these patients, 17 (11.2%) had a low enzyme activity (<3 kU/L), although the duration of apnea and DN were normal. Eight patients (5.3%) showed increased sensitivity to suxamethonium. Six heterozygotes (4%) had both normal and abnormal enzyme alleles with a medium range of apnea of 18.6 min (imitation range 12-35min) and their DN turned out to be less than normal (<80). Figure 1 shows the difference of the mean enzymatic activity between the two sexes.

Inhibition of enzyme activity by DN

The DN was determined after excluding two persons (1.3%) who were homozygotes and had an exceedingly low atypical enzyme allele (10% and 24%). The mean±SD DN value in the 149 out of 151 patients was 84.41±2.91%, and the DN ranged from 10-90% in these patients. Figure 2 reveals the mean±SD DN (percent) relative to enzyme activity (kU/L).

Apnea period

Two patients out of a total of 151 having a prolonged apneic period (240 and 360 min) were excluded from the study. The other 149 patients had an apnea period of 2.09-10.6 min. The mean apnea period was 6.37 min, and a standard deviation of 4.28 min was obtained. Figure 3 reveals the mean±SD apnea period and the related enzyme activities (kU/L).

Relationship between plasma cholinesterase (PChE) and factors responsible for apnea period and DN

Keeping Table I in consideration, a stronger relationship exists between plasma cholinesterase, apnea factors and dibucaine number. Seventeen patients in this study exhibited lower than normal enzymatic activity (≤3kU/L), nevertheless not all of them had prolonged apnea. The duration of apnea and DN in nine of these patients, however, was normal.

Eight of these patients in whom the DN was less than normal (<80%) exhibited a prolonged apnea period following succinylcholine administration (Table II).

DISCUSSION

The physiological activity of plasma cholinesterase has not been completely understood, except that it plays an important role in the rapid degradation of suxamethonium.

Suxamethonium remains the only agent capable of producing rapid, brief but intense paralysis, which is associated with a familiar myriad of potential problems. Both hereditary and acquired factors can cause a reduction in the enzyme's activity and by enhancing the patient's sensitivity to suxamethonium, eventually result in a prolonged period of apnea following the administration of suxamethonium.^{1,4,7,9}

In this study, the substrate propionylthiocholine and dibucaine solution are utilized for the specification of different kinds of enzymes. As shown in Fig. 1, it appears that enzyme activity is higher in men compared to women until the 6th decade of life, after which enzyme activity in women compared to men was highly prominent, as illustrated by only 6.8% reduction in enzyme activity in men compared to 14.6% reduction in women. These results are consistent with the results obtained elsewhere.^{4,10,11}

Pertaining to the plasma cholinesterase level and DN, Fig. 2 shows that the amount of DN is increased when there is an increase per unit in each enzyme group.^{12,13}

Figure 3 shows cholinesterase enzyme activity and the duration of apnea. A reduction in enzyme activity due to acquired or genetic factors increases the duration of apnea possibly because of enzyme involvement in the hydrolysis of suxamethonium. However, an increase in enzymatic activity above the normal range causes a 30% reduction in the duration of apnea.

It has become clear that with an accuracy of 0.001, a positive relationship exists between PChE and DN, meaning that an increased activity of the enzyme is associated with a simultaneous suppression of the enzyme by means of dibucaine. Regression equations strongly point towards this finding ($r=0.74$, $p<0.05$).

On the other hand, a negative relationship (-0.3530) with an accuracy of 0.001 is found between PChE and apnea, showing thereby that a reduction in the enzyme due to genetic or acquired factors causes an increase in duration of apnea following succinylcholine during anesthesia. Pearson's relationship favours this finding ($r=-0.71$, $p<0.05$). But a negative relationship (-0.9383) with an accuracy of 0.001 between DN and apnea is more important than a negative relationship between the duration of apnea and the activity of PChE.

It can be confirmed that it is the DN which can be utilized

to ascertain the sensitivity of a person to succinylcholine (suxamethonium) and estimation of the activity of the enzyme alone is not sufficient.

Looking at Table II, some patients had less than normal enzymatic activity (that is, 3 kU/L or less), nevertheless our patients did not display a prolonged duration of apnea except for persons whose DN was less than normal (<80%). This can be attributed to the fact that these persons were heterozygotes.

As only one inhibitor is employed in this study, we cannot unequivocally determine the genotype of patients but can infer the following conclusions. Subjects with a normal DN may have less than normal enzymatic activity (<3 kU/L), perhaps due to acquired factors or the fact that they might be heterozygotes with the normal gene having a maximum activity to cover up the deficiency of the abnormal gene.

There are many genetic variations of which many have been so far identified by different methods. Of these, there are some which exhibit an increased sensitivity to suxamethonium. Pseudocholinesterase or fresh frozen plasma has been administered often to speed recovery from prolonged succinylcholine block in atypical homozygotes. However, the relatively impure preparation of plasma cholinesterase currently available in Europe has never been marketed in the United States. Furthermore, administration of fresh frozen plasma nowadays is certainly not indicated for this purpose, in view of major concerns regarding the possible transmission of viral infections as a result of such treatment.¹⁴

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