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Fluoride Inhibition in Patients with Atypical Dibucaine-Resistant Plasmacholinesterase[1]

Sarla P. Kothary,[2] Kathleen B. Flynn,[2] and Elemér K. Zsigmond[2]

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The fluoride numbers (FN) of 144 individuals referred to our laboratory for dibucaine number (DN) determinations because of prolonged apnea following succinylcholine in the probands or relatives were determined by our new method using 2.5×10^{-4} M concentration of sodium fluoride at 37 C. Dibucaine-resistant homozygotes and heterozygotes both had lower FN than the normal homozygotes. A linear correlation between DN and FN for all these genotypes was found which is described by the equation $FN = 0.59DN + 32$. The correlation coefficient was $r = 0.94$. This equation will help in the correct identification of atypical fluoride resistant genotypes, since these ought to have an observed FN lower by 2 standard deviations than the FN calculated from this equation.

KEY WORDS: FN–DN correlation; fluoride-resistant plasmacholinesterase, dibucaine-resistant plasmacholinesterase.

INTRODUCTION

Genetic anomalies of the dibucaine-resistant ($E_1^aE_1^a$), the fluoride-resistant ($E_1^fE_1^f$), and the silent ($E_1^sE_1^s$) homozygous plasmacholinesterase genotypes are known to be responsible for the prolonged paralysis of the respiratory muscles (prolonged apnea) and cardiac arrhythmias following the intravenous injection of the usual 1.0 mg/kg dose of succinylcholine, the most widely used

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[2] University of Michigan Medical Center, Ann Arbor, Michigan 48109.

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muscle relaxant during anesthesia and surgery. The recognition of this anomaly and the genetic survey of the index family after the identification of the proband may circumvent untoward reactions to succinylcholine in the affected relatives (Harris and Whittaker, 1961; Foldes *et al.*, 1963).

Since the discovery of the fluoride-resistant plasmacholinesterase, many cases of fluoride-resistant heterozygotes ($E_1^uE_1^f$) and only a few homozygotes ($E_1^fE_1^f$) have been reported (Harris and Whittaker, 1961; Whittaker, 1967, 1968; Foldes *et al.*, 1963; Whittaker and Vickers, 1970; Thompson and Whittaker, 1966). In all these studies Na fluoride at a concentration of 5.0×10^{-5} M was used, which was claimed to inhibit normal plasma cholinesterase to about 64% while the fluoride-resistant homozygote was inhibited to less than 34%. Some of the reports on fluoride-resistant genotypes lacked the determination of DN, although some reported dibucaine numbers were also found abnormal in the so-called fluoride-resistant homozygotes and fluoride-resistant-silent heterozygotes. Moreover, the determination of the fluoride number (FN) alone could not differentiate between fluoride-dibucaine-resistant heterozygotes and fluoride-resistant homozygotes. The latter could only be differentiated by a normal DN.

The narrow range of percentages (64–34%) of inhibition in the various genotypes with the use of 5.0×10^{-5} M Na fluoride concentration at 25 C in contrast to the wide separation with the same concentration of dibucaine (15–75%) necessitated the development of a more specific method for the differentiation of the fluoride resistant phenotypes (Zsigmond, 1973). Because of the great dependence of the fluoride inhibition on temperature, we also increased the temperature from 25 to 37 C and increased the concentration of Na fluoride to 2.5×10^{-4} M, which gives 75% inhibition in a normal homozygote. We have been utilizing this modified method during the past 5 years to determine whether the fluoride inhibition may also be dependent on dibucaine inhibition. Therefore, both DN and FN were determined in a large number of dibucaine-resistant genotypes ($E_1^uE_1^a$ and $E_1^aE_1^a$).

MATERIALS AND METHODS

Between 1973 and 1978 a total of 144 plasma samples of patients with a history of prolonged apnea following succinylcholine administration and their blood relatives were sent to our laboratory for the determination of DN and FN.

The samples were stored at −20 C until the time of determination. All the experiments were carried out at 37 C and *p*H 7.4. Benzoylcholine chloride at a concentration of 5.0×10^{-5} M was used as a substrate for the determination of enzyme activity. Nupercaine hydrochloride at a concentration of 1.0×10^{-5} M and Na fluoride at a concentration of 2.5×10^{-4} M were used as inhibitors for the determination of DN and FN, respectively. All determinations were

carried out by a modification (Foldes *et al.*, 1960) of Kalow's ultraviolet spectrophotometric method using Beckman DU spectrophotometer at the wavelength of 240 nm.

RESULTS AND DISCUSSION

As shown in Table I, 50 individuals were normal homozygotes with mean ± SD DN of 76.0 ± 2.7 and FN = 77.0 ± 3.2. Fifty-four individuals were atypical normal heterozygotes with mean ± SD DN of 59.0 ± 7.4 and FN = 67.0 ± 5.4. The remaining 40 individuals were atypical dibucaine-resistant homozygotes with means ± SD DN of 18.0 ± 7.7 and FN = 43.0 ± 10.0. It is evident that the dibucaine-resistant heterozygotes and homozygotes also have abnormal fluoride inhibition or FN. There is a linear correlation between DN and FN with the equation shown in Fig. 1 ($r = 0.94$). So far we have not observed any plasma sample where FN would be 2 SD lower than DN. Consequently we were unable to identify any fluoride resistant heterozygote or homozygote, while we observed the expected incidence of dibucaine-resistant silent and usual genotypes in a surgical and anesthetized patient population (Whittaker and Vickers, 1970).

The original reports on the fluoride-resistant genotypes indicated a correlation between this genotype and prolonged apnea following succinylcholine administration (Harris and Whittaker, 1961; Whittaker, 1967; Whittaker and Vickers, 1970). Because of the paucity of the reported fluoride-resistant homozygotes with adequately documented prolonged apnea (Whittaker and

Table I. Correlation of DN with FN in Apneic Patients and Relatives With Various Plasmacholinesterase Genotypes

Genotype	BeCh HR[a]	DN	FN
$E_1^u E_1^u$	86.62	76.0 ± 2.68	77.0 ± 3.22
$N = 50$	± 30.06[b]	± 0.38	± 0.45
	± 4.25[c]		
$E_1^a E_1^u$	55.99	59.0 ± 7.41	67.0 ± 5.37
$N = 54$	± 23.59	± 1.00	± 0.73
	± 3.21		
$E_1^a E_1^a$	29.80	18.0 ± 7.66	43.0 ± 10.05
$N = 40$	± 11.71	± 1.21	± 1.59
	± 1.88		

[a] Benzoylcholine hydrolysis expressed as μmol/ml/hr.
[b] Standard deviation.
[c] Standard error.

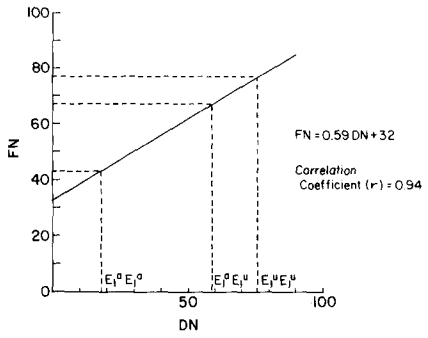

Fig. 1. Correlation of FN with Dn in various dibucaine-resistant genotypes. Note that the FN correlates linearly with the DN in all genotypes with a correlation coefficient of $r=0.94$. This equation can be utilized to predict the expected Fn in a dibucaine-resistant genotype. The observed FN should be lower by 2 SD deviation in a fluoride-resistant genotype than the value predicted from this equation.

Vickers, 1970) and because of the occurrence of prolonged apnea even in a large number of $E_1^uE_1^u$ (Foldes *et al.*, 1963; Zsigmond *et al.*, 1973), it is difficult to tell the clinical importance of fluoride-resistant genotypes. The only objective scientific way to establish a correlation between the abnormal genotype and prolonged neuromuscular block induced by succinylcholine is to determine the duration of neuromuscular block in patients with the fluoride-resistant PChE following intravenous succinycholine infusion.

CONCLUSION

The reported correlation equation will, it is hoped, facilitate the correct identification of the fluoride-resistant genotypes of PChE, thereby avoiding erroneous reporting of these genotypes in the future.

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