Laboratory Report

Stability of Pseudocholinesterase in Stored Blood

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No significant change in pseudocholinesterase levels was observed in random specimens of whole blood stored for as long as 30 days. Levels in plasma anticoagulated with heparin or ethylenediaminetetraacetic acid declined only slightly. Therefore, massive transfusions do not contraindicate succinylcholine administration. (Key words: Enzymes, cholinesterase; Transfusion, cholinesterase; Blood, cholinesterase.)

While no convincing link has been established between a clinical syndrome and deficiency of pseudocholinesterase per se, the activity of this enzyme can serve as an indicator of organophosphate exposure and is essential for the hydrolysis of therapeutically administered ester-type compounds such as succinylcholine. Since the half-life of pseudocholinesterase in vivo is only 3.4 days, and since pseudocholinesterase activity has been reported as being undetectable in blood stored for more than 24 hours, it might be assumed that the administration of succinylcholine to patients who have received massive transfusions should be avoided. The present study of the stability of pseudocholinesterase in vitro was undertaken to ascertain whether any such hazard exists.

Materials and Methods

Pseudocholinesterase activity was determined by a previously described modification of the technique of Ellman et al. Serum samples were preincubated with the sulphydryl-detecting reagent, 5,5′-dithiobis(2-nitrobenzoic acid), to eliminate the contribution of free protein sulphydryl groups. The activity of the enzyme was resistant to BW284C51, indicating the absence of erythrocytic acetylcholinesterase. Pseudocholinesterase activity is expressed as micromoles of acetylcholine hydrolyzed per minute per milliliter of plasma.

Blood samples were of two kinds. Specimens that had been collected from random donors and stored in the standard acid citrate dextrose anticoagulant were obtained from the State University Hospital Blood Bank. Erythrocytes were removed by centrifugation immediately prior to assay. Other samples were collected from three donors into Vacutainer tubes (Becton, Dickinson and Company, Rutherford, New Jersey) containing a variety of commonly used anticoagulants, or into empty tubes, where they were allowed to clot. Supernatant plasma or serum obtained by centrifuging these samples shortly after collection was stored under sterile conditions at 4°C until assayed.

Results

Pseudocholinesterase activities of samples of banked blood from random donors are shown plotted against durations of storage in figure 1. Mean activity was 0.935 units, with a standard deviation of ± 0.288 units. These values are consistent with those expected under the assay conditions of temperature and substrate concentration employed. The correlation coefficient for the least-squares line through the data points is ±0.0002, indicating that pseudocholinesterase is essentially stable under the conditions of storage at 4°C as used by our blood bank.
Fig. 1. Plasma pseudocholinesterase activities after storage for various periods under blood bank conditions. Assays were performed after removal of erythrocytes, and each point represents the mean of duplicate determinations on specimens from different donors.

Pseudocholinesterase activities in serum from uncouagulated blood specimens and in plasma from those collected into heparin, ethylenediaminetetraacetic acid or fluoride oxalate were determined after 0, 9, 18, 28, and 37 days of storage. The mean initial activity and rates of decline calculated from a least-squares line are shown in table 1. Only 5 to 10 per cent of the initial activity was lost after one month in the presence of the first two anticoagulants even though our conditions of storage were probably not as ideal as those in a blood bank. Fluoride-oxalate anticoagulation inhibited 63 per cent of the activity of pseudocholinesterase immediately, as expected, since fluoride is a known inhibitor of pseudocholinesterase.9

Discussion

Incidental references7,10 to observations that pseudocholinesterase activity in stored blood might decline rapidly are not in accord with the data that we present. Neither are they supported by the few other published studies of the stability of this enzyme under other conditions. When plasma is freed of erythrocytes, pseudocholinesterase levels do not diminish after storage at 0 C for at least five days11 and, possibly, for as long as several weeks.12,13 It is unlikely that this finding and our more relevant data on the stability of pseudocholinesterase in whole blood result from stabilization of the enzyme by a particular anticoagulant, since we also show preservation of activity in serum and in plasma anticoagulated with heparin or ethylenediaminetetraacetic acid. We conclude that administration of succinylcholine to patients who have received massive transfusions is not contraindicated.

Since pseudocholinesterase is essentially stable in whole blood under suitable conditions of in-vitro storage, factors other than intrinsic stability must be responsible for its relatively short half-life in vivo. Although relatively little is known about the mechanisms by which extracellular proteins are turned over, a similar phenomenon occurs in the case of serum albumin, a protein that is stable in vitro, but has a half-life of about six days in vivo.13

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Table 1. Loss of Pseudocholinesterase Activity from Anticoagulated Plasma at 4 C

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Initial Activity* (Units/ml)</th>
<th>Average Rate of Loss (Per Cent per 37 Days)</th>
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<tbody>
<tr>
<td>None</td>
<td>1.12</td>
<td>4.73</td>
</tr>
<tr>
<td>Heparin</td>
<td>1.03</td>
<td>8.26</td>
</tr>
<tr>
<td>EDTA†</td>
<td>0.860</td>
<td>10.5</td>
</tr>
<tr>
<td>Fluoride-oxalate</td>
<td>0.305</td>
<td>-41.3</td>
</tr>
</tbody>
</table>

* Mean for three donors.
† Ethylenediaminetetraacetic acid.
References

Obstetric Anesthesia

METHOXYFLURANE AND LABOR Three groups of obstetric patients were examined to evaluate the effect of methoxyflurane on plasma fluoride concentration. Fifty patients received 0.2–0.5 per cent methoxyflurane in 50–70 per cent N₂O for analgesia during delivery. Durations of exposure were 4–41 minutes. Forty-one patients received methoxyflurane (approximately 0.3 per cent) by self-administration during labor. Analgesia during delivery was provided as in the first group. Durations of exposure were 18–120 minutes. Seventeen patients undergoing cesarean section received 0.2–0.5 per cent methoxyflurane in 50–70 per cent N₂O (eight prior to deliver and six only after delivery). Durations of exposure were 23–75 minutes. In patients receiving methoxyflurane for delivery the peak plasma fluoride concentrations was 6 µmol/l. In parturients receiving methoxyflurane for labor and delivery, peak fluoride concentration was 10 µmol/l, while peak concentration following cesarean section was 22 µmol/l. In one patient receiving methoxyflurane for the vaginal delivery of twins, the total time of administration was 105 minutes while delivered concentrations were as high as 0.8 per cent. Her peak level was 73 µmol/l. The data indicate that normal labor and delivery as well as cesarean section may be carried out with careful administration of methoxyflurane without fear that biologically significant concentrations of fluoride will result. However, in the occasional patient (especially following treatment with certain drugs) excessively high concentrations of fluoride may result. (Palahnik RJ, Cumming M: Plasma fluoride levels following obstetrical use of methoxyflurane. Canad Anaesth Soc J 22:291–297, 1975.)