Reversal of Granulocyte Adherence to Nylon Fibers Using Local Anesthetic Agents: Possible Application to Filtration Leukapheresis

By Charles A. Schiffer, Frances T. Sanel, Virginia B. Young, and Joseph Aisner

The effects of the cationic anesthetic agents tetracaine and lidocaine on granulocyte function, morphology, and adherence to nylon fibers were studied in an attempt to improve current methods of granulocyte collection by filtration leukapheresis (FL). When dissolved in acid-citrate-dextrose (ACD) plasma, these drugs significantly increased granulocyte elution from the fibers in a dose-related fashion. Granulocytes exposed to tetracaine and lidocaine remained more than 95% viable, retained normal bactericidal capacity after the drugs were washed from the cells, and had preserved membrane integrity, as evidenced by the normal ultrastructural appearance of tetracaine-exposed cells and an absence of leakage of lysozyme or lactic dehydrogenase. Granulocytes eluted with the anesthetic agents were rounded in shape with a reduction in the number of filopodial cytoplasmic projections and a relative absence of cytoplasmic vacuolization when compared to granulocytes eluted with ACD plasma alone. Dose-related inhibition of phagocytosis and adherence, which was largely reversible after washing the granulocytes, was noted. Greater than 95% of the lidocaine could be removed from the eluate with a single centrifugation and resuspension, indicating that granulocytes prepared by FL with anesthetic-enhanced elution could be potentially transfusable.

It has been known for many years that granulocytes will avidly adhere to a variety of surfaces. The mechanism of adherence is still poorly understood, however, particularly when compared to the progress which has been made in other aspects of granulocyte physiology. Recently, attention has been focused on the process of granulocyte adherence because of increased clinical utilization of granulocytes obtained by reversible adhesion to nylon fibers. Filtration leukapheresis (FL), as originally described by Djerassi et al.1 and modified by Herzig et al.2 and Buchholz et al.3 has considerable appeal because of the high yield of granulocytes obtained and the relatively low cost of the collection system.

Although granulocytes obtained by FL are of definite clinical value in the treatment of infection in neutropenic patients,4,5 it is clear that these granulocytes are altered both morphologically and functionally by the collection procedure. Cytoplasmic vacuolization,4,6-10 partial degranulation,6,9 variable alterations in chemotaxis8 and in vitro bactericidal capacity,4,8 decreased in vivo recovery,4,11,12 and a significant increase in recipient transfusion reactions4,11,13...
have all been reported by several investigators. It is likely that granulocytes are
damaged during the process of adhesion and release and that this damage is
exacerbated by the physical force of “tapping” the nylon fiber filters.8 10 This
“tapping” is necessary in order to elute the granulocytes from the fibers ef-
ficiently.3

Recent reports have noted that cationic anesthetic agents could inhibit and
reverse the adhesion and spreading of macrophages or sarcoma cells on the sur-
face of plastic petri dishes.14 15 The effects of these membrane-active drugs were
associated with a reduction in the number of plasmalemmal projections from
the cells. These changes were completely reversible after washing the cells and
removing the drugs. The purpose of the present study was to determine whether
granulocyte adherence could be reversed using similar pharmacologic manipu-
lations. Both tetracaine and lidocaine were studied as prototype agents, the
latter being of interest because it would be potentially transfusable if these
pharmacologic techniques were ever applied to FL. In addition, the effects of
these drugs on granulocyte morphology and function were assessed.

MATERIALS AND METHODS

Adherence and Elution
In order to study the effects of a large number of variables on granulocyte elution, a simple
system was designed which permitted accurate and repetitive measurement of granulocyte ad-
herence and release: 200 mg of nylon fiber obtained from Leukopaks (Fenwal, Morton Grove, Ill.)
were tightly packed to a volume of 1 ml in a tuberculin syringe. Then 10 ml of heparinized blood
(3 units heparin/ml), obtained from normal volunteers who were taking no medications, were
pumped through the fibers at a rate of 1 ml/min using a constant infusion pump (Harvard
pump, Harvard Apparatus Co., Dover, Mass.). The syringe containing the blood (or subsequent
eluting solutions) was tightly attached to the tuberculin syringe by placing a loop of rubber tubing
over the tip of the former and inserting this into the top of the tuberculin syringe. White blood
cell counts and differentials were done on the original sample and the eluate, allowing for calcu-
lation of both the percentage adherence and the absolute number of granulocytes which adhered.
All experiments were carried out at room temperature in duplicate and all blood counts were
done electronically with a Coulter Model SR (Coulter Electronics, Hialeah, Fla.).

Elution experiments were then done testing different concentrations of tetracaine (0.5 - 2.0 mM)
and lidocaine (5 15 mM) dissolved in normal saline, heparinized plasma, Hank’s balanced salt
solution (HBSS), acid citrate dextrose (ACD) plasma and citrate phosphate dextrose (CPD)
plasma. After the fibers were “loaded” by passage of 10 ml of heparinized blood, 20 ml of eluting
solution were immediately pumped without tapping through the fibers at a rate of 1 ml/min.
ABO-identical plasma was used in the plasma experiments. The percentage elution was calculated
by comparing the number of granulocytes actually eluted with the number of granulocytes from
the original 10 ml of blood which had adhered to the fibers. Experimental results were compared
to studies conducted simultaneously using control solutions without the cationic anesthetic.

In later experiments, 300 400 ml of whole heparinized blood were passed by gravity through
standard Leukopaks at a rate of approximately 50 ml/min. After 10 - 15 min, adherent granulocytes
were eluted without tapping by passing 500 700 ml of 1.5 mM tetracaine in ACD plasma, 15
mM lidocaine in ACD plasma, or ACD plasma alone by gravity at a rate of approximately 10 - 15
ml/min at room temperature. The percentage elution was calculated and the eluted granulocytes
were evaluated morphologically and for phagocytic ability as outlined below.

Effect on Granulocyte Function and Morphology
Phagocytosis. One ml of whole heparinized blood was exposed to varying concentrations of
tetracaine and lidocaine for 30 min at room temperature. An excess (20 μl) of 0.81-μm latex par-
ticles (Bactolatex, Difco, Detroit, Mich.) was then added, the mixture was incubated for 30 min
at 37°C with gentle tumbling, and smears were made and stained with Wright’s stain. A crude phagocytic index was obtained by counting the number of granulocytes which had ingested 0, 1-4, or >4 latex particles as compared to control cells. Granulocytes eluted from the large Leukopaks were washed once and resuspended in autologous heparinized plasma, and then were incubated with latex particles as described above.

**Adherence.** Ten ml of whole heparinized blood was mixed for 30 min at room temperature with varying concentrations of lidocaine and tetracaine and pumped through fiber-packed syringes as described above. The percentage adherence was then compared to control cells.

**Bactericidal capacity.** The bactericidal capacity against *Staphylococcus aureus* by granulocytes exposed to 1.5 mM tetracaine and 15 mM lidocaine was assessed using the colony-counting method of Quie et al.16 Granulocytes separated from heparinized blood by gravity sedimentation were exposed to the drugs for 60 min at room temperature. The granulocytes were then centrifuged at 180 g for 8 min, washed 3 times in saline, resuspended in HBSS, and incubated with gentle rocking for 2 hr at 37°C with *S. aureus*. Control granulocytes were handled identically except that they were not exposed to the drugs.

**Lysozyme and lactic dehydrogenase (LDH) release.** These activities were measured as indicators of possible granulocyte injury after exposure to tetracaine and lidocaine. Granulocytes from four ABO-identical donors were initially separated by dextran sedimentation of ACD-anticoagulated blood. Excess red blood cells were lysed by the addition of 2 volumes of 0.8%, ammonium chloride to 1 volume of the granulocyte-rich plasma for 1 min at 4°C. The solution was then centrifuged at 80 g for 10 min and the supernatant was removed. The cell pellet was washed twice in ABO-identical ACD plasma obtained from a single donor, resuspended in ACD plasma, and adjusted to a final count of 6 × 10⁶ granulocytes/ml. Tetracaine (final concentrations 1.5 and 2 mM) and lidocaine (15 and 20 mM) were added to 1 ml of the granulocytes and mixed on a rocker for 60 min at room temperature. The mixture was then centrifuged at 1600 g for 5 min and lysozyme content was measured in the supernatant using a turbidometric assay and lyophilized *Micrococcus lysodeikticus* as substrate17 (Lysozyme Assay Kit, Worthington Biochemical Corp., Freehold, N.J.). Supernatant LDH was measured by a colorimetric determination using pyruvate as substrate18 (Sigma Chemical Co., St. Louis, Mo.). Maximal enzyme release was determined by repetitive freeze thawing of control granulocytes in both the LDH and lysozyme studies.

**Viability and morphology.** Granulocytes separated from heparinized and ACD-anticoagulated blood by gravity sedimentation and exposed to varying concentrations of tetracaine and lidocaine for 60 min at room temperature were examined on Wright’s stained slides and wet mount preparations. Viability was determined by trypan blue dye exclusion. Experimental and control slides were coded and evaluated without knowledge of the source of the specimens.

**Electron microscopy.** On two occasions granulocytes collected by gravity sedimentation of heparinized blood were examined by electron microscopy after exposure to 1.5 mM tetracaine at room temperature for 30 min. In addition, granulocytes eluted from the nylon fibers with 1.5 mM tetracaine in ACD plasma as described above were compared with granulocytes eluted using ACD plasma alone. Granulocytes were pelleted at 150 g for 8 min prior to fixation at 25°C with Karnovsky solution diluted 1:1 with cacodylate buffer using previously described techniques.9 The granulocytes were also evaluated for glycogen content and distribution as previously described.9

Crystalline tetracaine hydrochloride (Sigma) dissolved in distilled water or autologous plasma was used in the tetracaine experiments; lidocaine hydrochloride was supplied as preservative-free Xylocaine (4%, solution; Astra Pharmaceutical Products, Worcester, Mass.).

In all experiments, a paired study design was employed in which blood from the same donors was used in the control and drug-exposed experimental groups. Results were compared statistically using the Student's t test for paired observations.

**RESULTS**

**Viability**

Granulocytes exposed for 60 min to 1.5 mM tetracaine or 15 mM lidocaine (0.4%,) remained >98%, viable as assessed by trypan blue exclusion. Viability decreased to 90%, 95%, with 20 mM lidocaine and was as low as 80% in some experiments with 23 mM lidocaine (0.6%, pH 6.8, in ACD plasma). Although
normal viability was maintained using 2 mM tetracaine, membrane distortion with broad surface protrusions was noted in many of the granulocytes. Results were similar using ACD- or heparin-anticoagulated blood. Based on these observations, maximal concentrations of 15 mM lidocaine and 1.5 mM tetracaine were used in the elution experiments and in most of the other studies.

Granulocyte Elution

In preliminary experiments on 26 volunteers the percentage granulocyte adherence was 77% ± 3% (SEM) and duplicate samples agreed within ± 2%. Furthermore, only 11.7% ± 2.8% (SEM) of circulating lymphocytes, which are largely nonadherent cells, were trapped, indicating that active adherence by granulocytes had taken place rather than nonspecific trapping in the interstices of the fibers.

Less than 10% of adherent granulocytes were eluted from the tuberculin syringe system by heparinized plasma, HBSS, normal saline or CPD plasma (pH 7.3), and elution was not increased by the addition of 1.5 mM tetracaine or 15 mM lidocaine to these solutions. ACD plasma alone (pH 6.9–7.1) eluted 10%–15% of granulocytes. Elution could be further increased by the addition of tetracaine or lidocaine to the ACD plasma. As shown in Fig. 1, enhanced elution with lidocaine appeared to be pH dependent, increasing considerably at pH 6.7–6.8 (p < 0.01 compared to ACD plasma alone); 15 mM lidocaine in ACD plasma had a pH of 6.9–7.0, and the lower pH solutions were achieved by the addition of extra ACD (approximately 1–2 ml of ACD/50 ml of plasma) to the original ACD plasma. It should be noted that this additional ACD served to increase elution efficiency itself, although always to a lesser extent than with the lidocaine plasma (Fig. 1). This pH influence was probably related to an alteration in the degree of ionization of the lidocaine·HCl with a resultant effect on the ability of the drug to interact with the cell membrane. Ten mM lidocaine·ACD plasma failed to increase granulocyte elution compared to controls, while 12.5 mM lidocaine·ACD plasma (69% elution at pH 6.6) was somewhat less effective than the 15-mM concentrations. Elution with tetracaine·ACD plasma (pH 6.9–7.1) was also dose related, increasing significantly only at tetracaine concentrations of 1.5 and 2 mM (Table 1). The final osmolality of

![Fig. 1. Statistically significant (p < 0.05) enhancement of elution seen with lidocaine·ACD plasma at pH 6.7–6.8 and pH 6.6](image-url)
Table 1. Granulocyte Elution With Tetracaine-ACD Plasma

<table>
<thead>
<tr>
<th>Tetracaine Concentration (mM)</th>
<th>Control ACD Plasma</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution (%)</td>
<td>17.2 ± 6.5*</td>
<td>26.8 ± 6.2</td>
<td>24.7 ± 4.2</td>
<td>51.2 ± 10.1†</td>
<td>62 ± 6.9†</td>
</tr>
<tr>
<td>(Range)</td>
<td>(1-42)</td>
<td>(11-50)</td>
<td>(17-44)</td>
<td>(29-81)</td>
<td>(45-89)</td>
</tr>
</tbody>
</table>

*Mean ± SEM of six experiments.  
†p < 0.025 compared to controls.

the lidocaine and tetracaine plasma solutions ranged between 290 and 300 mOsm.

In two experiments using the standard Leukopaks, ACD plasma alone (pH 6.9) eluted 14% of adherent granulocytes, as compared to 64% and 51% (mean 58%) for 1.5 mM tetracaine-ACD plasma (pH 7.0) and 46% and 47% with 15 mM lidocaine-ACD plasma (pH 6.8).

Granulocyte Function and Morphology

**Phagocytosis.** In six experiments, 95% of control granulocytes ingested >4 latex particles. There was no effect on phagocytosis by 0.5 mM and 1.0 mM tetracaine, a moderate reduction by 1.5 mM tetracaine (83% of granulocytes with >4 particles, range 52-100%), and a marked reduction with 2 mM tetracaine (3%, with >4 particles, 80% with no ingestion). To determine whether the drug effect was reversible, whole blood exposed to tetracaine for 30 min was centrifuged at 180 g for 4 min and the buffy coat was removed, washed once in heparinized plasma, resuspended in heparinized plasma, and incubated with latex particles.

In six experiments phagocytosis returned to control levels after removal of 1.5 mM tetracaine (98% of cells with >4 particles) and improved toward normal with 2 mM tetracaine (80% of cells with >4 particles, range 52-100%). Results with lidocaine were similar, except that considerable inhibition of phagocytosis occurred with the lower concentration of 5 mM (41% with >4 particles), while almost complete inhibition was seen at 15-20 mM concentrations. As in the tetracaine experiments, phagocytosis returned toward normal after washing (57% with >4 particles with 5-15 mM versus 88% for controls). The incomplete reversal after washing may be related to the persistence of small amounts of lidocaine after a single wash and resuspension, although a possible residual toxic effect of the higher concentrations seems more likely.

Phagocytosis of latex particles and *S. aureus* by granulocytes eluted with lidocaine and tetracaine from the large Leukopaks was equivalent to controls after washing and resuspension in heparinized plasma (>95% of cells ingested >4 particles).

**Adherence.** As shown in Table 2, there was a dose-related decrease in the ability of granulocytes to adhere to nylon fibers after exposure to tetracaine and lidocaine. The inhibitory effect by lidocaine was noted with concentrations as low as 5 mM.

In two experiments, buffy coat preparations were exposed to tetracaine (1.5 and 2.0 mM) and lidocaine (15 and 20 mM) for 30 min, washed, resuspended in autologous heparinized plasma, and pumped through the tuberculin syringe
Table 2. Anesthetic Effect on Granulocyte Adherence

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Control</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adherence (%)</td>
<td>83.2 ± 6.8*</td>
<td>77 ± 10.8</td>
<td>66.6 ± 9.6</td>
<td>59 ± 12.4†</td>
<td>52.8 ± 9.9†</td>
</tr>
<tr>
<td>(Range)</td>
<td>(65–100)</td>
<td>(43–96)</td>
<td>(35–93)</td>
<td>(32–100)</td>
<td>(29–79)</td>
</tr>
<tr>
<td>Lidocaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adherence (%)</td>
<td>78.3 ± 4.7</td>
<td>46 ± 5.5†</td>
<td>35.3 ± 5.2†</td>
<td>40.7 ± 2.7†</td>
<td>16.3 ± 8.5†</td>
</tr>
<tr>
<td>(Range)</td>
<td>(69–84)</td>
<td>(35–52)</td>
<td>(25–42)</td>
<td>(37–46)</td>
<td>(3–32)</td>
</tr>
</tbody>
</table>

*Mean ± SEM of five experiments with tetracaine and three with lidocaine.
†p < 0.05 compared to control.

system. Adherence improved to normal after washing the tetracaine- and 15 mM lidocaine-exposed cells, but remained subnormal (53%) in the 20 mM lidocaine experiments.

Bactericidal capacity. In three experiments there was no difference in the percentage of organisms killed by granulocytes exposed to tetracaine (mean kill 95°, range 94°–97°) and lidocaine (mean 93°, range 90°–96°) when compared to controls (mean 95°, range 91°–98°).

Lysozyme and LDH leakage. Preliminary experiments demonstrated that the addition of tetracaine or lidocaine did not affect the standard reference curves for LDH or lysozyme. There was no significant leakage of enzymes with various concentrations of tetracaine and lidocaine (Table 3). The final pHs were 6.8–6.9 in the lidocaine studies and 6.9–7.1 in the tetracaine experiments.

Morphology. Granulocytes eluted from the tuberculin syringe and Leukopak systems by either ACD plasma alone, 15 mM lidocaine, or 1.5 mM tetracaine in ACD plasma were >95° viable. There was, however, a distinct difference in morphology on wet mount preparations. Multiple filopodial extensions, cytoplasmic blebs with polarization of the nucleus to one side of the cell, and decreased granule movement were seen in the majority of the granulocytes eluted with ACD plasma. These changes were particularly apparent at pH 6.6. In contrast, granulocytes eluted with tetracaine- or lidocaine–ACD plasma had smoother surfaces and fewer cytoplasmic blebs, and appeared to be “rounded” in shape. On Wright's stained slides, cytoplasmic vacuolization was present in approximately 50% of the ACD plasma–eluted cells, but was seen rarely in the drug-eluted cells. In addition, “smudged” cells were considerably more prominent on smears of the ACD plasma–eluted cells, suggesting increased fragility of these granulocytes.

Table 3. Lysozyme and LDH Leakage

<table>
<thead>
<tr>
<th>Supernatant lysozyme (μg/ml)</th>
<th>Freeze-Thaw</th>
<th>Control</th>
<th>Tetracaine Concentration (mM)</th>
<th>Lidocaine Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
<td>2.0</td>
<td>15 20</td>
<td></td>
</tr>
<tr>
<td>Supernatant lysozyme</td>
<td>15.8 ± 1.9*</td>
<td>6.9 ± 1</td>
<td>7.2 ± 0.3 6.9 ± 0.4</td>
<td>6.6 ± 0.4 6.6 ± 0.5</td>
</tr>
<tr>
<td>(μg/ml)</td>
<td>(12.5–21)</td>
<td>(4.5–9)</td>
<td>(6.6–8)  (5.8–7.5)</td>
<td>(5.3–7.2)  (5.5–8)</td>
</tr>
<tr>
<td>Supernatant LDH</td>
<td>1376 ± 17E</td>
<td>488 ± 17</td>
<td>450 ± 13 466 ± 13</td>
<td>441 ± 13 392 ± 20</td>
</tr>
</tbody>
</table>

*Values represent the mean ± SEM of four experiments with the ranges in parentheses. There are no statistically significant differences between controls and any of the experimental values.
Fig. 2. (A) Electron micrograph of tetracaine-exposed granulocytes. Number and distribution of cytoplasmic granules appear normal. Plasma membranes are relatively smooth and intact and cytoplasmic elements are comparable to normals. Chromatinic loops (N) were not uncommon in tetracaine-treated granulocytes. Golgi elements (G) are prominent and elongated mitochondria with dense matrices (arrows) are present. × 9500. (B) Light micrograph of adjacent 1-μm section from the same block stained with toluidine blue. The granulocytes are rounded with smooth surfaces. × 1480.

When examined by electron microscopy, granulocytes exposed to tetracaine were relatively normal in morphology, with smooth surfaces, occasional broad cytoplasmic outpouchings, and preservation of normal numbers of granules (Fig. 2). In other electron micrographs (not shown), glycogen deposits were normal in quantity and distribution. Granulocytes eluted with ACD plasma
Fig. 3. (A) Granulocytes eluted with ACD plasma alone. Multiple microvillous (f) and filopodial (ff) processes and complex surface loops devoid of cytoplasmic elements were characteristic of these preparations. Cortical vacuoles (v) are numerous; granules appear to be diminished. × 10,000. (B) Light micrograph of 1-μm section from the same block. × 1550.
alone (Fig. 3A and B) were characterized by increased surface activity with multiple thin, angular, microvillous projections devoid of cytoplasmic organelles. Considerable cytoplasmic vacuolization was observed in many of these cells. In contrast, granulocytes eluted with tetracaine-ACD plasma (Fig. 4A and B) tended to be rounded in shape with smooth surfaces. Some cytoplasmic

Fig. 4. (A) Granulocytes eluted with tetracaine-ACD plasma. Rounded cells exhibit broad lamellopodia (L) containing cytoplasmic organelles. Microvilli or cortical vacuoles were rarely present. Prominent Golgi (G); elongated, dense mitochondria (arrows). × 10,000. (B) At higher magnification the dense matrix and disordered cristae of the mitochondria and intact granules can be seen. × 54,000.
projections were also present in these cells, but these protrusions were invariably broad based, short, and blunted and usually contained multiple cytoplasmic organelles. The number and distribution of granules and glycogen appeared normal in these cells and cytoplasmic vacuolization was uncommon. There appeared to be an increased number of nuclear chromatinic bridges and fine chromatinic loops in the granulocytes exposed to tetracaine (Fig. 2A). Moderate numbers of markedly elongated mitochondria with dense matrices and slightly disordered cristae were present (Figs. 2 and 4), and in many cells Golgi saccules were enlarged and distended (Figs. 2 and 4). These observations suggest that tetracaine penetrated the cell and affected intracellular structures.

**Elution following filtration leukapheresis.** After the above results were obtained, four elution experiments after filtration leukapheresis were performed in which one of the filters was eluted with 15 mM lidocaine-ACD plasma without tapping, while the other filter was eluted with the standard ACD plasma-saline solution with tapping. The granulocytes were then collected in separate bags and comparative yields and morphology were assessed. Granulocyte yields per filter were similar using the two different elution procedures (control mean 13.5 x 10^9, range 6.7-19.3; lidocaine 12.6 x 10^9, range 5.6-17.7). As described previously, the granulocytes collected with tapping were vacuolated and exhibited membrane deformity when examined on wet mounts. The lidocaine-eluted cells were rounded in shape, similar to those seen using the tuberculin syringe system, and, after washing and resuspension in HBSS, phagocytized latex particles and S. aureus normally (>90%, with >4 particles). In three experiments, the lidocaine-collected granulocytes were centrifuged at 180 g for 8 min and resuspended in ABO-compatible ACD plasma after the supernatant was removed. Lidocaine determinations (kindly performed by Dr. Philip R. Reid, Johns Hopkins School of Medicine) were then done on the final product and the supernatant and revealed that >95% of the lidocaine had been removed by this simple maneuver.

**DISCUSSION**

The use of a simple "miniaturized" model for granulocyte adherence facilitates the study of a large number of variables affecting granulocyte adherence and release. The present study demonstrates that the cationic local anesthetic agents, lidocaine and tetracaine, both alter granulocyte adherence to nylon fibers and enhance elution of granulocytes from the fibers. The effect on elution was dose related and occurred only when these drugs were dissolved in ACD plasma. The latter observation suggests that it was also necessary to chelate divalent cations before efficient elution could occur, and, indeed, increased elution was also seen when additional ACD alone was added to the plasma. Local anesthetics can alter membrane binding of calcium and affect calcium flux across plasma membranes and mitochondria, and it is perhaps these properties which result in the "additive" effect produced when mixed with ACD. The failure to increase elution when dissolved in CPD plasma (pH 7.2) suggests that lower pHs were necessary to allow interaction of the drugs with the granulocytes, possibly by increasing the lipid solubility of the anesthetics. Acidic pH was not sufficient to enhance elution, however, because lidocaine dis-
solved in HBSS (final pH 6.8) and normal saline (pH 6.6) was ineffective as an eluting agent.

The differences in the shape and morphology of eluted granulocytes on the wet mount preparations and electron micrographs suggest that the mechanism of granulocyte release from the fibers may be somewhat different for ACD and the cationic anesthetic agents. Granulocytes either exposed to or eluted by lidocaine and tetracaine were rounded in shape with an absence of filamentous cytoplasmic projections. In this regard, the granulocytes were similar to the rounded shape of the sarcoma cells and macrophages exposed to anesthetics as described by Rabinovitch and DeStefano. In contrast, granulocytes eluted with ACD had increased membrane activity and multiple fine filopodia.

We have previously shown, using scanning electron microscopy, that granulocytes adherent to nylon fibers are flattened on the fibers and exhibit multiple microvillous and filopodial cytoplasmic projections binding the cells to the fibers. It is possible that the anesthetic agents may induce withdrawal of these projections with subsequent release from the fibers, while elution with ACD is associated with persistence of at least some of the membrane projections. Although the morphology of the ACD-eluted granulocytes was improved compared to granulocytes we have examined prepared by FL, the granulocytes eluted with ACD plasma without tapping still demonstrated increased vacuolization and probable increased fragility, as indicated by the large number of smudged cells on the stained smears. Wright et al. have demonstrated that granulocyte morphologic and functional abnormalities are increased with more prolonged adherence of the granulocytes to the fibers. The present study demonstrates, however, that even granulocytes which adhered to fibers for a minimal period of time seem to exhibit some damage when eluted with ACD plasma alone.

The cellular site at which the anesthetic agents act to reverse adherence is not known. Rabinovitch and DeStefano have shown that preincubation of macrophages with colchicine does not prevent anesthetic-induced cell shape change, indicating that the anesthetic effect may not be mediated by alteration of microtubules. Additional experiments also have suggested that the shape change is not related to anesthetic-induced inhibition of transmembrane sodium conductance. These investigators have postulated that the effect of these lipid-soluble drugs may be mediated by alterations in the fluidity of the plasma membrane or by a metabolic inhibition resulting in changes in intracellular ATP levels. In this regard, it is possible that the atypical mitochondria seen in our electron micrographs (Fig. 4) are a morphologic correlate of this postulated metabolic inhibition. Our electron micrographs did not permit a quantitation of the microtubules and microfilaments in the tetracaine-exposed granulocytes, but centriole-associated granulocyte microtubules and cortical bands of microtubules in platelets were seen and appeared normal. The significance of the tetracaine effect on the Golgi apparatus is as yet unclear.

A variety of granulocyte functions are inhibited by the cationic anesthetics, including phagocytosis, adherence and changes in cell shape. Cullen and Haschke have also shown that latex-stimulated oxygen consumption and nitroblue tetrazolium dye reduction are inhibited by lidocaine, while Giddon and
Lindhe have shown, using an in vivo hamster cheek pouch model, that topically applied lidocaine can block the adherence of circulating granulocytes to the endothelium of venules. In this in vivo system, as well as in the studies of Rabinovitch and DeStefano and our phagocytosis and adherence experiments, the drug-induced inhibition was largely reversible after the cells were washed and the anesthetic removed.

It is premature at this time to recommend that tetracaine or lidocaine be utilized clinically as an adjunct to FL. At higher drug concentrations, and particularly with lidocaine, toxic effects were noted which could conceivably become even more apparent with increased duration of exposure. In addition, studies utilizing more sophisticated functional tests which compare anesthetic-eluted granulocytes with control cells obtained after routine leukapheresis are necessary. Other classes of compounds are also of potential interest. Two groups of investigators have shown that increased levels of cyclic 3',5'-adenosine monophosphate (cAMP) reduce granulocyte adhesion, and that cyclic nucleotide inducers such as atropine and prostaglandin E, can decrease adhesion. Further experiments with these compounds as well as other anesthetic agents should be pursued in an attempt to improve the quality of granulocytes obtained by FL and to further our understanding of cell adherence and release.

ACKNOWLEDGMENT

The authors wish to thank Ronald Clawson, Robin Hartman, and Theda Strohecker for their excellent technical assistance and Ira M. Goldstein, M.D., for his helpful suggestions.

REFERENCES


Reversal of granulocyte adherence to nylon fibers using local anesthetic agents: possible application to filtration leukapheresis

CA Schiffer, FT Sanel, VB Young and J Aisner