Comparative Analysis Between Dextran Sulfate Adsorption and Direct Adsorption of Lipoproteins in their Capability to Reduce Erythrocyte Adhesiveness/Aggregation in the Peripheral Blood

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Abstract: The purpose of this study was to compare the degree of erythrocyte adhesiveness/aggregation (EAA) reduction of two low-density lipoprotein (LDL) apheretic procedures, namely direct adsorption of lipoproteins (DALI) and dextran sulfate adsorption (DSA). A significant ($P < 0.001$) reduction of EAA was noted in six hypercholesterolemic patients who underwent a total of 40 apheretic sessions and no difference was noted in the degree of EAA reduction by the two techniques. Thus, being a real-time and point-of-care test, the erythrocyte adhesiveness/aggregation test can be applied in relevant situations of acute ischemia, where therapeutic LDL apheresis could improve the hemorheology of individuals with increased concentrations of cholesterol and inflammatory sensitive proteins. Key Words: Erythrocyte adhesiveness/aggregation test, Low-density lipoprotein apheresis.

It has been repeatedly shown that increased red blood cell aggregation might have detrimental effects on the microcirculation (1–6). A rapid and effective measure to reduce this increased tendency of the cells to adhere and aggregate might, therefore, be of clinical relevance (7). In this context, a simple, cheap and real-time methodology to determine the state of erythrocyte adhesiveness/aggregation in the peripheral blood before and following therapeutic interventions might be of interest.

We have previously shown that a significant reduction in the state of erythrocyte adhesiveness/aggregation in the peripheral venous blood can be obtained by applying plasmapheresis to individuals with hypercholesterolemia (8). The present study reports on the results that were obtained with two therapeutic low density lipoproteins (LDL) apheretic methods: dextran sulfate adsorption (DSA) and direct adsorption of lipoproteins (DALI). The findings of this study are relevant for the immediate identification of individuals with an ischemic event, high fibrinogen and cholesterol concentrations, who might benefit from an LDL apheresis approach.

MATERIALS AND METHODS

Patients and treatment protocol

We included six patients in the study. All of them gave their consent for participation in the study that was approved by the local ethics committee. The age, types of hyperlipidemia, and concomitant diseases and medications in four study patients for whom a direct comparative analysis between both DSA and DALI systems were performed are given in Table 1. Two additional patients who underwent LDL apheresis using DSA system only were included: a 30-year-old female with homozygous familial hypercholesterolemia (FH), and a 62 year old female with primary hyperlipidemia type 2. None were smokers. The diagnosis of FH homozygosity was made by LDL receptor analysis. Patient 3 was diagnosed as FH heterozygous according to accepted clinical criteria.
(xanthelasma, LDL cholesterol concentrations above the ninety-fifth percentile, and compatible family history). Secondary hyperlipidemia was excluded in all the six patients.

All patients were already maintained on a regular LDL apheresis treatment prior to study entry. Familial hyperlipidemia homozygosity was the indication for LDL apheresis in the three study patients with homozygous FH. Patient 3, who is FH heterozygous, did not respond satisfactorily to combined hypocholesterolemic treatment for 1 year before LDL apheresis was begun (the lowest LDL cholesterol level was 180 mg/dL). Of the two study patients with type 2 primary hyperlipidemia, the indications for LDL apheresis were failure to achieve target levels of LDL cholesterol under maximal hypocholesterolemic treatment in one patient and in the other patient, there was severe hepatic enzyme elevation during treatment with various statins. All the patients were on a strict dietary modification based on the American Heart Association Step-2 recommendations.

Patients 1, 2, 3, and 4, underwent four consecutive treatments with each of the LDL apheresis systems tested (DSA and DALI). The other two study patients underwent four consecutive LDL apheresis treatments with DSA only. The interval between consecutive treatments was between 5 and 17 days. Blood was drawn at baseline and at the termination of each procedure. In the other two patients, the access was through an arteriovenous fistula. It should be emphasized that the blood samples at the termination of the procedure were taken from the arteriovenous fistula after the removal of 10 mL of blood (as dead space).

**Low-density lipoprotein apheresis systems**

**Dextran sulfate cellulose adsorption**

Low-density lipoprotein apheresis was performed using an automated system equipped with the dextran sulfate cellulose column of 400 mL as an adsorbent (Liposorber LA-40, Kaneka Corporation, Osaka, Japan). The columns were disposable and not intended for reuse. The column was prerinsed with 1000 mL of Ringer’s solution containing 2000 IU of heparin. Plasma was obtained by centrifugation of whole blood, using a continuous flow COBE Spectra apparatus (Gambro BCT, Lakewood, Co, USA). Anticoagulation during the procedure was achieved using acid citrate dextrose formula A (ACD-A) combined with the heparin (3000 IU heparin with 500 mL ACD-A). Acid citrate dextrose formula A was mixed with the patient’s blood at a ratio of 1:28. In each treatment, one plasma volume was treated. This was calculated from 8% of the body weight (blood volume) and corrected by the hematocrit.

**Direct adsorption of lipoproteins**

The elimination of apolipoprotein (apo) B containing lipoproteins (LDL and Lp(a)) from whole blood was performed by electrochemical adsorption onto polyacrylate-coated polyacrylamide beads (DALI 750 mL adsorber, Fresenius 4008 ADS, Germany). The adsorber columns were prerinsed with 6000 mL of priming solution, in order to prevent serum cations absorption. Heparin (2000 IU) was added to the first 2000 mL of priming solution as anticoagulant. During each treatment a fixed volume of 4000 mL whole blood was processed.

**Laboratory variables**

The whole blood count was performed using an automatic cell analyzer ADVIA 120 Hematology System (Bayer Diagnostics, Dublin, Ireland) and the erythrocyte sedimentation rate was calculated using the method of Westergren (9), while quantitative fibrinogen determinations were performed by using the ACL PT-based method and Futura ACL Coagulometer (Instrumentation Laboratory, Milano, Italy). Total cholesterol and triglyceride concentrations were determined on a Roche/Hitachi 747 Analyzer.

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**TABLE 1. Clinical details and medications of the four study patients for whom a direct comparative analysis between both dextran sulfate adsorption and direct adsorption of lipoproteins systems was performed**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex (M/F)</th>
<th>Type of hyperlipidemia</th>
<th>Concomitant diseases</th>
<th>Medical treatment of hyperlipidemia</th>
<th>Concomitant drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>F</td>
<td>Homozygous FH</td>
<td>IHD, AS, carotid stenosis</td>
<td>Atorvastatin 40 mg</td>
<td>Aspirin, Beta blockers</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>M</td>
<td>Homozygous FH</td>
<td>IHD</td>
<td>Atorvastatin 40 mg</td>
<td>Aspirin</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>F</td>
<td>Heterozygous FH</td>
<td>HTN</td>
<td>Atorvastatin 40 mg</td>
<td>Aspirin, Ca channel blockers, Disothiazide, HRT</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>M</td>
<td>Type-2 primary hyperlipidemia</td>
<td>IHD</td>
<td>None</td>
<td>Aspirin, Nitrates, AT II antagonists</td>
</tr>
</tbody>
</table>

M, male; F, female; FH, familial hypercholesterolemia; IHD, ischemic heart disease; AS, aortic stenosis; HTN, hypertension; HRT, hormone replacement therapy.
(Roche Diagnostics, Mannheim, Germany), with a Raichem kit (Reagents Applications Inc., San Diego, California, USA). High-density lipoprotein cholesterol was assayed on Roche/Hitachi 747 Analyzer, with a Randox kit (RANDOX Laboratories, Crumlin, UK). Low-density lipoprotein cholesterol was assayed on the Roche/Integra 400 Analyzer (Roche Diagnostics, Mannheim, Germany) by a direct method, after specific detergent treatment on the other cholesterol-containing particles. Apolipoprotein A-1 and Apolipoprotein B were assayed on the Roche/Integra 400 Analyzer by an immunoturbidimetric method, using specific antisera. Lipoprotein(a) was assayed on the Roche/Integra 400 Analyzer by a particle-enhanced immunoturbidimetric method using latex particles coated with anti-Lp(a) antibodies.

**The erythrocyte adhesiveness/aggregation test**

Blood was obtained before and following LDL apheresis into a syringe containing sodium citrate 3.8% (one volume of citrate and three volumes of whole blood). One drop of blood was applied onto a glass slide that was kept at an angle 30°, as described elsewhere (10,11). Five minutes later the slide was scanned in an image analyzer (INFLAMET; Inflamet, Tel Aviv, Israel) (12). The degree of erythrocyte adhesiveness/aggregation in the peripheral blood is proportional to the vacuum radius (VR), which is a measurement of the size of the spaces that are formed between the aggregated red blood cells.

**Statistical analysis**

The difference between pre- and post-procedure was calculated for each patient in each one of the four actions, in each method.

The two methods were compared as follow. Paired t-test was used for the group of patients that were treated in both methods. Independent t-test was applied to compare all the actions performed in one method to those performed in the second method. A one-way analysis of variance was used to compare between the four different actions assuming independence of the four actions. This analysis was separated to each method.

The statistical significance level was set to 0.05 and the SPSS (SPSS Inc., Chicago, IL, USA) for windows software, version 11.0, was used for the analysis.

**RESULTS**

The current group comprised four patients with hypercholesterolemia who underwent four sessions of DSA LDL apheresis and four sessions of DALI apheresis each (total 32 apheresis sessions), and two patients who underwent four sessions of DSA LDL apheresis only (total 8 apheresis sessions). The results of the laboratory variables of rheological relevance and those of the erythrocyte adhesiveness/aggregation test for the patients who underwent the two different LDL apheresis procedures are reported in Tables 2 and 3. A significant (P < 0.01) reduction in the degree of erythrocyte adhesiveness/aggregation (as determined by using the vacuum radius variable) was noted following both LDL apheretic procedures (Table 2). A significant reduction was also noted for other variables of hemorheologic relevance, including ESR and fibrinogen (Table 2 and Fig. 2), and for lipid variables (Table 3). A paired t-test study showed no significant difference comparing the two methods for these reductions. A representative picture obtained in one of our patients is given in Fig. 1.

Finally, in order to exclude a rebound effect in cholesterol concentrations we calculated the difference between pre- and post-procedure cholesterol concentrations and divided this number by the time (days) elapsed between the procedures. No difference in the rebound effect was noted between the two different LDL apheresis procedures (data not shown).

**DISCUSSION**

Previous studies have shown the potential detrimental hemorheological effects that increased red blood cell aggregation might have regarding capillary

**TABLE 2. Reduction of erythrocyte adhesiveness/aggregation (expressed as the VR) and other variables of hemorheological significance following single LDL apheresis in the 4 patients who underwent dextran sulfate adsorption (DSA) and direct adsorption of lipoproteins (DALI)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>DSA Before</th>
<th>DSA Following</th>
<th>% Reduction</th>
<th>DALI Before</th>
<th>DALI Following</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm/h)</td>
<td>26 ± 10</td>
<td>9 ± 8</td>
<td>68 ± 23*</td>
<td>31 ± 20</td>
<td>13 ± 12</td>
<td>60 ± 18*</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>478 ± 110</td>
<td>372 ± 96</td>
<td>22 ± 10*</td>
<td>493 ± 167</td>
<td>383 ± 135</td>
<td>22 ± 10*</td>
</tr>
<tr>
<td>Vacuum radius (microns)</td>
<td>18.6 ± 14.3</td>
<td>3.5 ± 1.4</td>
<td>70 ± 23*</td>
<td>15.8 ± 15.5</td>
<td>5.2 ± 6.1</td>
<td>64 ± 19*</td>
</tr>
</tbody>
</table>

In one patient ESR determination was not available following DSA procedure. *P < 0.01. ESR, erythrocyte sedimentation rate. VR, vacuum radius. Values given as mean ± standard deviation.
slow flow (1–5) and tissue oxygenation (6). Increased erythrocyte aggregation has been shown to be present in hypercholesterolemic patients (13) and various therapeutic apheretic procedures have been undertaken to attenuate this tendency of cell aggregation (14–17).

The present study focused on the ability of two therapeutic LDL apheretic procedures, DSA and DALI, to reduce the state of erythrocyte adhesiveness/aggregation in the peripheral blood. This comparative analysis was done so as to clarify whether either of those approaches has an advantage regarding its potential to reduce the aggregability of the cells. The results of the study are significant in that they show that both methods have the same effect on the state of adhesiveness/aggregation of the cells following the apheretic procedure.

Increased erythrocyte aggregation in hypercholesterolemic patients could contribute to the appearance of vascular occlusion. The increased cell aggregability can be attenuated by the administration of HMG-CoA inhibitors (18,19) but also by the application of apheretic procedures (20). The beneficial effects of apheretic procedures might be related to the reduction of lipid fractions, but an additional benefit can stem from the reduction in the tendency of the red blood cells to stick to each other and aggregate. In this regard, it might be relevant to determine whether both commonly used LDL atheretic approaches (DSA and DALI) have the same efficacy. Our study shows that this is indeed the case. Compared with our previously reported data regarding plasmapheresis (8), the results look similar in terms of reducing the degree of erythrocyte adhesiveness/aggregation.

The erythrocyte adhesiveness/aggregation test is based on the notion that multiple inflammation sensitive proteins including fibrinogen, gamma globulins, ceruloplasmin, haptoglobin, α1 antitrypsin and even C-reactive protein are involved in the induction and/or maintenance of enhanced erythrocyte aggregability (21–24). We are using the red blood cell as an autologous sensor for the detection of enhanced concentrations of these inflammation sensitive elements. Therefore, instead of looking at the concen-

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**TABLE 3. Reduction of lipid variables following single LDL apheresis in the 4 patients who underwent dextran sulfate adsorption (DSA) and direct adsorption of lipoproteins (DALI)**

<table>
<thead>
<tr>
<th>Lipid Variable</th>
<th>DSA Before</th>
<th>DSA Following</th>
<th>% reduction</th>
<th>DALI Before</th>
<th>DALI Following</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>369 ± 106</td>
<td>175 ± 72</td>
<td>51 ± 12*</td>
<td>359 ± 90</td>
<td>160 ± 50</td>
<td>55 ± 8*</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>258 ± 93</td>
<td>109 ± 59</td>
<td>38 ± 11*</td>
<td>264 ± 76</td>
<td>94 ± 42</td>
<td>64 ± 9*</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>51 ± 12</td>
<td>36 ± 11</td>
<td>32 ± 10*</td>
<td>53 ± 16</td>
<td>36 ± 13</td>
<td>31 ± 9*</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>147 ± 38</td>
<td>91 ± 38</td>
<td>40 ± 16*</td>
<td>150 ± 38</td>
<td>79 ± 31</td>
<td>47 ± 20*</td>
</tr>
<tr>
<td>Lipoprotein(a) (mg/dL)</td>
<td>8.9 ± 9</td>
<td>4.0 ± 4</td>
<td>54 ± 8*</td>
<td>9.2 ± 9.6</td>
<td>3.0 ± 3</td>
<td>66 ± 11*</td>
</tr>
<tr>
<td>Apo a (mg/dL)</td>
<td>14.5 ± 3.9</td>
<td>12.3 ± 3.5</td>
<td>15 ± 7*</td>
<td>14 ± 3.6</td>
<td>12 ± 3</td>
<td>13 ± 6*</td>
</tr>
<tr>
<td>Apo b (mg/dL)</td>
<td>21.9 ± 7</td>
<td>9.3 ± 4.2</td>
<td>57 ± 10*</td>
<td>21 ± 6</td>
<td>7.8 ± 3</td>
<td>63 ± 9*</td>
</tr>
</tbody>
</table>

*P < 0.01. LDL, low-density lipoprotein; HDL, high-density lipoprotein; Apo a, apolipoprotein a; Apo b, apolipoprotein b. Values given as mean ± standard deviation.
trations of individuals proteins before and following each apheretic procedure (which is neither practical nor cheap), we suggest looking at the summarized effect that these proteins have on the degree of erythrocyte adhesiveness/aggregation. Although similar information could be obtained by using the Westergren sedimentation rate, the erythrocyte adhesiveness/aggregation might be more informative (25). In addition, the fact that the results can be obtained within a couple of minutes, suggests a novel method of monitoring the LDL apheretic treatment in terms of hemorheological efficiency. This point might be especially relevant for those conditions in which the procedure is applied to patients with an acute ischemic event. In fact, a recent study showed that LDL apheresis is especially effective in individuals with hyperfibrinogenemia (7). This protein is a major determinant of erythrocyte aggregation (23) and has been shown to correlate significantly with the degree of erythrocyte adhesiveness/aggregation in the peripheral blood (26).

We conclude that both DSA and DALI apheretic procedures reduce the degree of erythrocyte adhesiveness/aggregation to a similar degree. Thus, in terms of hemorheology, they might express the same efficiency. Being simple, rapid and cheap, the erythrocyte adhesiveness/aggregation test might be an attractive bedside methodology to determine biochemical effects of rheological relevance once LDL apheretic procedures are applied in clinical situations of acute ischemia (7).

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**REFERENCES**