Plasma Exchange and Low Density Lipoprotein Apheresis in Watanabe Heritable Hyperlipidemic Rabbits

Masakatsu Kano, Junji Koizumi, Arvind Jadhav, and Gilbert R. Thompson

Comparison of the effects of plasma exchange using lipoprotein-deficient plasma with those of LDL apheresis using a dextran sulphate column was undertaken in two groups of Watanabe heritable hyperlipidemic rabbits pre-labelled with \(^{3}H\)-cholesterol. Total and HDL cholesterol were reduced more by plasma exchange but HDL cholesterol rebounded higher after LDL apheresis; rises in HDL cholesterol correlated with preceding decreases in both HDL and total cholesterol. An increase in the specific activity of HDL cholesterol occurred on the day after each procedure, being more marked after plasma exchange, and was accompanied by a decrease in the cholesterol/phospholipid ratio of HDL. These results suggest that an influx of extravascular HDL into plasma occurred after both procedures, resulting in mobilization of tissue cholesterol. (Arteriosclerosis 7:256–261, May/June 1987)

Plasma exchange, which was first introduced over 10 years ago to treat homozygous familial hypercholesterolemia (FH), results in gradual regression of cutaneous and tendon xanthomata.\(^1\)\(^2\) Indirect evidence that this reflects mobilization of tissue cholesterol was obtained by pre-labelling patients' tissues with radioactive cholesterol and demonstrating acute rises in the specific activity of plasma cholesterol after plasma exchange.\(^1\) In one instance analysis of individual lipoproteins showed this rise to be localized to high density lipoprotein (HDL) cholesterol.\(^3\)

An inverse relationship between the concentrations of HDL cholesterol in plasma and the size of the exchangeable pool(s) of tissue cholesterol has been proposed\(^4\) and the possible role of HDL in transporting cholesterol from tissues into plasma was reviewed recently.\(^5\) Several,\(^6\)\(^7\) but not all,\(^8\) epidemiological surveys have shown an increased risk of developing coronary heart disease (CHD) in those with low HDL cholesterol levels, and angiographic data suggest that HDL cholesterol is inversely correlated with the severity of coronary arterial lesions.\(^10\)\(^11\) as well as with the likelihood that these will progress.\(^12\)\(^13\)

In the light of these findings and because plasma exchange removes HDL as well as low density lipoprotein (LDL), techniques designed to conserve HDL during LDL removal have been developed. These include exposing whole blood to heparin-agarose beads\(^14\) and perfusing plasma through affinity columns containing LDL antibodies\(^15\) or dextran sulphate.\(^16\) Each method specifically removes LDL but not HDL, a process known collectively as LDL apheresis. The present study compares the effects of plasma exchange and LDL apheresis in Watanabe heritable hyperlipidemic (WHHL) rabbits, an animal model of FH.\(^17\)

Methods

WHHL rabbits were bred locally from homozygous males and heterozygous females, kindly provided by Dr. Y. Watanabe. The progeny were classified as heterozygotes or homozygotes according to their serum cholesterol levels while on a normal diet; those with a value above 100 mg/dl at 3 months were regarded as homozygotes, the remainder as obligate heterozygotes. The former remained on a normal diet but heterozygotes received supplements that increased the cholesterol content of their diet to 0.2% for the 4 months prior to the start of the study. During this period blood was obtained from an ear vein each month for determination of serum cholesterol, and only heterozygotes with cholesterol levels that had risen into the homozygote range were used. The body weight of the rabbits used in these studies was 3.5 to 4.5 kg. All were female.

Each rabbit received an intravenous injection of (1α, 2α-\(^{3}H\)) cholesterol (Amersham International, United Kingdom) 100 to 600 \(\mu\)Ci, which was first dissolved in ethanol and then mixed with 0.5 ml of Lipostabil (Natterman, Cologne, West Germany) and 2 ml of autologous plasma, at 15 to 22 days before undergoing plasma exchange or LDL apheresis. This interval was chosen after a preliminary study had shown that the specific activity of cholesterol in the plasma of a normcholesterolemic New Zealand white (NZW) rabbit became lower than that of skin, adipose tissue, muscle and red blood cells by 2 to 3 weeks after the injection of \(^{3}H\)-cholesterol. At 21 days the specific activity of cholesterol in liver was similar to plasma but that in the aorta was much lower (Figure 1).

Prior to undertaking plasma exchange, blood was ob-
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Changes in specific activity of cholesterol in plasma, adipose tissue, and skin of a normal New Zealand white rabbit following an injection of 3H-cholesterol. The specific activities of cholesterol in kidney, muscle, liver, and aorta at the time of sacrifice on Day 21 are also shown.

Blood samples were obtained at intervals before and after each procedure for determination of the concentration and specific activity of total and HDL cholesterol in plasma, and also the concentration of total and HDL phospholipid. HDL was obtained by adding 0.4% phosphotungstic acid and 2 mM MgCl₂ to plasma immediately after this had been separated from fresh blood samples at 4°C, followed by centrifugation and filtration of the turbid supernatant. Measurement of HDL cholesterol by this method gave mean ± SE values of 19.8 ± 7.1 and 7.0 ± 0.5 mg/dl respectively in six NZW and six WHHL rabbits compared with values of 18.9 ± 3.9 and 7.6 ± 3.1 mg/dl obtained by assaying the infranatant of samples ultracentrifuged at d = 1.063. Cholesterol was measured by GLC and phospholipid by enzymatic assay (Wako Chemicals, West Germany). Radioactivity was determined in a Beckman LS 1801 liquid scintillation counter, after dissolving 0.05 to 0.2 ml of sample in 10 ml Beckman Ready Solve MP. Tissue samples were extracted with chloroform/methanol, 2:1 (vol/vol) and the chloroform phase was separated, dried down, and saponified before determination of specific activity.

Results

The possibility that the lipoprotein particles present in the plasma of cholesterol-fed rabbits pass through the plasma separator less easily or adsorb to the LA 01 column less well than those in the plasma of WHHL homozygotes was catered for by constructing two similar groups of rabbits, each comprising four homozygotes and two cholesterol-fed heterozygotes.

Changes In Plasma Cholesterol Concentrations

The effects of plasma exchange and LDL apheresis on serum total and HDL cholesterol concentrations are shown in Figure 2.

Mean values of serum total and HDL cholesterol were similar in the two groups before each procedure although levels tended to be higher in cholesterol-fed heterozygotes than in homozygotes. Percentage decreases in total cholesterol were slightly greater after plasma exchange than after LDL apheresis (66% and 59%, respectively), despite the larger volume of plasma processed during the latter procedure (see Table 1). As expected, the percentage
Table 1. Experimental Details

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Weight (kg)</th>
<th>Plasma processed (ml)</th>
<th>Heparin given (I.U.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL apheresis</td>
<td>WHHL Hmz* (4)</td>
<td>4.05</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>WHHL CF-Htzf (2)</td>
<td>4.05</td>
<td>140</td>
</tr>
<tr>
<td>Plasma exchange</td>
<td>WHHL Hmz* (4)</td>
<td>3.75</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>WHHL CF-Htzf (2)</td>
<td>4.1</td>
<td>120</td>
</tr>
</tbody>
</table>

*Watanabe heritable hyperlipidemic rabbit homozygote, †cholesterol-fed heterozygote.

decrease in HDL cholesterol was much more marked after plasma exchange (62%) than after LDL apheresis (28%).

Sequential changes in total and HDL cholesterol during the week after each procedure are shown in Figures 3 and 4. The greater decrease in total cholesterol after plasma exchange was followed by a faster rate of rebound than after LDL apheresis (58 mg/dl/d versus 32 mg/dl/d). In contrast, although HDL cholesterol fell less after LDL apheresis, the subsequent rise above baseline on Day 5 exceeded that seen after plasma exchange (27% versus 8%).

Rises in HDL cholesterol concentration after each procedure, expressed as the difference between immediate post-procedure and subsequent peak values, correlated well with preceding decreases in total and HDL cholesterol, as shown in Figure 5. Correlation coefficients were higher when increments in HDL cholesterol were correlated with previous decreases in HDL cholesterol (B) rather than total cholesterol (A) but the differences were slight.

Changes in Specific Activity

The effects of LDL apheresis on the specific activities of total and HDL cholesterol in plasma are illustrated in Figure 6. The specific activity of HDL cholesterol became higher than that of total cholesterol during the first few days

Figure 3. Changes in plasma total cholesterol after LDL apheresis and plasma exchange. Mean ± SE values are shown relative to the pre-exchange values, including the average level during the week before each procedure.

Figure 4. Changes in HDL cholesterol after LDL apheresis and plasma exchange, expressed in a manner similar to Figure 3.

after injection of 3H-cholesterol and rose even more after LDL apheresis. Similar changes occurred after plasma exchange, but to an even more marked extent, as shown in Figure 7. The rise in the relative specific activity of HDL cholesterol was evident within 2 hours after plasma exchange but was not apparent until 6 hours after LDL apheresis.

Figure 5. Correlations between the rebound in HDL cholesterol in Watanabe heritable hyperlipidemic homozygotes (circles) and cholesterol-fed heterozygotes (triangles) after plasma exchange (PE, open symbols) or LDL apheresis (APH, closed symbols) and the preceding decrease in HDL or total cholesterol. The correlation coefficients (r) for each procedure are shown.
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Figure 6. Effect of LDL apheresis on specific activity of plasma total and HDL cholesterol in a Watanabe heritable hyperlipidemic homozygous rabbit injected with \(^3\)H-cholesterol 21 days previously.

Changes in HDL Composition

Changes in HDL composition, as judged from the cholesterol/phospholipid ratio, occurred after both plasma exchange and LDL apheresis. However, baseline HDL composition differed markedly between homozygotes and cholesterol-fed heterozygotes and this, rather than the type of procedure undergone, was the major determinant of subsequent changes in composition.

As shown in Table 2 the mass ratio of cholesterol/phospholipid in HDL was much higher in cholesterol-fed heterozygotes than in homozygotes. Decreases became evident on the first and second days after plasma exchange or LDL apheresis, the change being relatively slight in homozygotes but quite marked in cholesterol-fed heterozygotes. Changes in this ratio on Day 1 were negatively correlated with changes in HDL specific activity, when each variable was expressed as a percentage of its pre-exchange value \((r = -0.68)\), as shown in Figure 8. Ratios returned to near their initial values between the third and fifth days after either procedure.

Discussion

These studies set out to compare the respective capabilities of plasma exchange and LDL apheresis to induce mobilization of tissue cholesterol. The greater reduction in total cholesterol achieved by plasma exchange suggests that it is more efficient than a single LA 01 column at removing LDL from a given volume of plasma. This presumably reflects the limited capacity of the column to bind cholesterol, which is estimated at 1 g per 100 ml of adsorbent. However, LA 01 columns do not bind HDL, the small reductions in the latter which were observed being

Table 2. HDL Cholesterol/Phospholipid Mass Ratios after Plasma Exchange or LDL Apheresis

<table>
<thead>
<tr>
<th>Time</th>
<th>Homozygotes (n = 4)</th>
<th>Cholesterol-fed heterozygotes (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.18 ± 0.02</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td>6 hours</td>
<td>0.18 ± 0.03</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>1 day</td>
<td>0.17 ± 0.02</td>
<td>0.35 ± 0.07</td>
</tr>
<tr>
<td>2 days</td>
<td>0.16 ± 0.02</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>3 days</td>
<td>0.18 ± 0.03</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>5 days</td>
<td>0.20 ± 0.02</td>
<td>0.46 ± 0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
due to losses of whole plasma during the filling and emptying of the extracorporeal circuit. Evidence that HDL plays a role in transporting tissue cholesterol and the knowledge that more HDL is lost during plasma exchange than during LDL apheresis suggested that the latter procedure might be the more advantageous. This likelihood was strengthened by observations that LDL apheresis of whole blood using heparin-agarose or of plasma using an immuno-affinity column results in an eventual increase in HDL cholesterol after each procedure. On the other hand, the proven ability of plasma exchange to induce regression of xanthomata27 and, possibly, atheroma25 implies that it possesses considerable cholesterol-mobilizing ability, despite the loss of HDL entailed.

Administration of radioactive cholesterol has long been used to label tissue cholesterol in both humans and experimental animals.27 Radioactivity in plasma equilibrates rapidly with cholesterol in liver and red cells, more slowly with fat and muscle, and least rapidly with the arterial intima.38 By waiting long enough, it is possible to ensure that all tissues other than brain and arteries will have a higher specific activity than plasma. Under these circumstances, any subsequent rise in plasma specific activity must indicate movement of cholesterol from tissues into plasma, due either to exchange or net transfer. If the rise follows maneuvers that do not entail the introduction into plasma of unlabelled cholesterol, then it can be assumed to indicate net transfer rather than exchange. This phenomenon was observed in WHHL rabbits both after plasma exchange with LPDP, which contains only very small amounts of cholesterol, and after LDL apheresis, suggesting that both procedures caused a net efflux of tissue cholesterol into plasma. Rises in HDL specific activity, which were maximal at 24 hours, were more marked after plasma exchange than after LDL apheresis, possibly reflecting the greater reduction of the intravascular pool of HDL. However, it is unlikely that much of the mobilized cholesterol came from arteries since turnover of cholesterol there is so much slower than in most other tissues, including xanthomata.36,38 Similar findings have been observed after plasma exchange in humans1,3 and pigs and after fasting in obese subjects.31

The rise in HDL specific activity during the 2 days after each procedure was accompanied by a reduction in the cholesterol/phospholipid ratio of HDL. Both findings are compatible with the entry into plasma of HDL derived from the extravascular compartment, in that the HDL of peripheral lymph has a higher phospholipid content than HDL in plasma and has been reported to be the major acceptor of free cholesterol derived from tissues.33

The mechanism whereby plasma exchange with LPDP induces efflux of tissue cholesterol could relate to its content of very high density lipoprotein and apo A-I, extrapolating from data obtained using human lipoprotein deficient serum (LPDS) in vitro.34,35 However, LDL apheresis did not involve administration of LPDP, but despite this, the rebound in HDL cholesterol on Day 5 was even more marked than after plasma exchange. In both instances the magnitude of the overall increases in HDL cholesterol during the 5 days after each procedure were proportional to the preceding decreases, which were greater after plasma exchange. The likelihood that removal of small amounts of HDL by LDL apheresis stimulates HDL synthesis has been discussed by Parker et al.24 If true, it would be expected that HDL synthesis would be stimulated to an even greater extent by plasma exchange, although direct evidence of this will be hard to obtain, owing to the non-steady-state conditions pertaining.

If LDL apheresis and plasma exchange do indeed promote movement of HDL into plasma from the extravascular compartment, this could be secondary to the reduction in non-HDL cholesterol that accompanies these procedures, since the rebound in HDL cholesterol was proportional to preceding decreases in not only HDL but also total cholesterol, comprising mainly very low and low density lipoproteins. Recent data suggest that HDL is bound to cells by specific receptors, the expression of which is directly proportional to cellular cholesterol content.36 Depletion of cellular cholesterol by maneuvers such as LDL apheresis and plasma exchange might be expected to reduce receptor-mediated binding of HDL in tissues and thus promote the movement of extravascular HDL back into plasma. Whether postprocedural rises in HDL reflected the presence of apo E-rich particles was not determined.37

On the basis of the evidence presented here, plasma exchange seems comparable to LDL apheresis in its ability to reduce total cholesterol and thus promote efflux of tissue cholesterol, despite the loss of HDL entailed. However, the procedure was tolerated less well by WHHL rabbits than LDL apheresis, and early indications are that this preference also applies to man. For this and other reasons, such as cost and safety, it seems likely that LDL apheresis may eventually supplant plasma exchange in the treatment of most forms of severe hypercholesterolemia.

References
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