Lipoprotein Composition and Transport in the Pig and Dog Cardiac Lymphatic System

PIERRE JULIEN, EUGENE DOWNAR, AND AUBIE ANGEL

SUMMARY The cardiac lymphatic system of pigs was found to consist of valved efferent lymph vessels draining into a cardiac lymph node, which is similar to the lymphatic system found in dog and man. The interstitial lipoproteins of the heart were studied by sampling cardiac efferent lymph in anesthetized fasting pigs (n = 4) and dogs (n = 5). In pigs, the lymph flow rate was 2.0 ± 0.7 ml/hr (mean ± SRM) and in dogs, 1.2 ± 0.3 ml/hr. The total cholesterol concentration was 54.9 ± 8.0 and 33.8 ± 6.3 mg per 100 ml in cardiac lymph of pigs and dogs, respectively. The ratio of lymph:plasma cholesterol differed in the two species: 0.82 in pigs and 0.46 in dogs. More than 90% of cardiac lymph cholesterol was recovered in high density lipoprotein (HDL) and in low density lipoprotein (LDL) in both pigs and dogs. In pigs, LDL was the major cholesterol-carrying lipoprotein in plasma and lymph, whereas in canine plasma and lymph, this was HDL. The appearance of 125I-LDL in canine cardiac lymph following iv administration was rapid and reached a plateau by 1 hour. The half appearance of 125I-LDL in lymph was 5 minutes compared to half clearance of plasma (rapid phase) of 44 minutes. The lymph:plasma ratio of LDL specific activity was 0.48 ± 0.15, suggesting continuous addition of cold LDL in the interstitial space. The high concentration and turnover of cholesterol-rich lipoprotein in cardiac lymph suggest that the predilection of the coronary bed for atherosclerotic degeneration may be a function of the interstitial lipoprotein concentration and composition as well as the plasma concentration of lipoprotein species.


PIGS are particularly useful in the study of cardiovascular disease (Detweiler, 1966) because of the many similarities between pig and human hearts and great vessels (Lumb, 1966) and the comparable physical and chemical properties of pig and human very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) (Mahley and Weisgraber, 1974a). Furthermore, LDL is the major cholesterol-carrying lipoprotein in swine (Mahley et al., 1975), as in humans (Fredrickson et al., 1978), and arteriosclerosis may be induced by dietary cholesterol feeding (Mahley et al., 1975).

A significant proportion of LDL and HDL is metabolized and degraded by extravascular, extrahepatic tissues. Sniderman et al. (1975) demonstrated that after iv administration of 125I-LDL in hepatectomized pigs, the plasma clearance of 125I-LDL was unimpaired and subsequently reported that a significant portion (~50%) was distributed in compartments other than the liver. In studies on lymph from the human foot, Reichl et al. (1975) identified three major antigens of plasma VLDL, LDL, and HDL and showed that the LDL was functionally active (Reichl et al., 1978).

The cardiac lymph system is of particular interest because this fluid is thought to closely represent the interstitial compartment and would more closely reflect metabolic events at the cell interface. In the present study, we outlined the cardiac lymphatic system in swine and then analyzed cardiac efferent lymph in pigs and dogs, to determine the heart's interstitial lipoprotein cholesterol content. Cardiac lymph contained high concentrations of cholesterol in lipoproteins composed mainly of LDL and HDL particles. The ratio of LDL:HDL cholesterol in cardiac lymph was 10-fold greater in pigs than in dogs, indicating marked species differences. Following iv administration of 125I-LDL, we demonstrated rapid appearance of 125I-LDL in cardiac lymph. However, the specific activity of lymph LDL did not equilibrate with that of plasma LDL, suggesting addition of unlabeled LDL in the extravascular space.

Methods

Anatomy of Cardiac Lymphatics

Eighteen healthy male Yorkshire pigs weighing 15-40 kg were studied. They had been fed a commercial diet (Pig Growena no. 2, Purina) for 4 weeks. The animals were restrained, and general anesthesia was induced with intravenous (ear) 2.5% sodium pentobarbital (1 ml/kg), the trachea was intubated, and animals were maintained with flu- ethane:O2. Respiration was controlled with a Harvard respirator connected to a cuffed intratracheal tube. A median sternotomy was performed and the anterior wall of the pericardial sac was incised. Evans blue (0.1 ml of T1824, 0.5% aqueous soln) or Patent blue (2.5% saline soln) was injected.
epicardially into both ventricles, care being taken to ensure no contamination of pericardial fluid and of the lymphatic system of the parietal pericardium. The blue-stained efferent lymph vessels and lymph nodes were identified and dissected. Finally, in 15 of the 18 pigs, the heart and lymph nodes were excised, and diagrams of the anatomical pathways were made immediately.

For light microscopic investigation, the efferent lymph trunk was dissected in vivo in three pigs. The distal and proximal ends of the lymph trunk were ligated simultaneously to avoid a collapse of the lymph vessel during excision and fixation. The tissue was fixed overnight in buffered (4%) formaldehyde:(1%) glutaraldehyde, pH 7.2 (McDowell and Trump, 1976). After they had been washed in 0.1 M phosphate buffer at pH 7.2, tissues were post-fixed in 1% osmium tetroxide, washed again in phosphate buffer, and dehydrated in ethanol. Pieces of tissues were embedded in epoxy resin (Anderson and Andre, 1968). Semi-thin sections were stained with 1% toluidine blue for light microscopic examination.

**Sampling of Cardiac Lymph**

Four healthy Yorkshire pigs weighing 22-30 kg and five healthy mongrel dogs weighing 20-27 kg were fasted for 24 hour and anesthetized as described above. Through a thoracotomy, a main efferent cardiac lymph trunk, identified by direct inspection without the aid of dye so as to prevent contamination of lymph, was cannulated with a polyethylene catheter (Clay Adams) 0.28 mm i.d. x 0.61 mm o.d., caudal to the cardiac lymph node. The polyethylene catheter was siliconized by immersion in Siliclad (Clay Adams) prior to use to prevent clotting of lymph. Correct placement of the catheter was confirmed at the end of each experiment by intracardiac injections of Evans blue. Lymph was collected by continuous gravity drainage for 2-4 hours into tubes immersed in crushed ice and containing 50 µl EDTA (7.5%) as an anticoagulant. Midway through the experiment, blood was withdrawn by venopuncture into Vacutainer tubes containing 7.5% EDTA. Care was taken to prevent contamination of cardiac lymph with blood by avoiding traumatic manipulations and injury of mediastinal tissues. Thus, the animals were not anticoagulated, intramyocardial dye injections were avoided, except to validate the lymph drainage route, and the pericardial sac was not incised until the end of the experiment. Microhematocrit centrifugation of lymph confirmed the absence of red blood cells, as no visible sediment occurred.

**Analysis of Cardiac Lipoproteins**

Plasma and cardiac lymph lipoproteins were analyzed by agarose electrophoresis (Beckman agarose gel kit) before centrifugation at density 1.006 for 18 hours. Following centrifugation of pig plasma at density 1.006, the supernatant (d < 1.006) was removed by tube slicing and, to 2 ml of the infranatant fraction (d > 1.006) containing LDL and HDL, 0.2 ml of 1 M MnCl₂ containing 450 U of heparin (Sigma) (NIH Manual, 1974) were added to precipitate the LDL. To isolate LDL, HDL, and HDL₂ fractions from canine plasma and lymph, whole plasma and lymph initially were centrifuged at density 1.006 for 18 hours and the infranatant (d > 1.006) obtained by tube slicing was recentrifuged sequentially following stepwise increase in density to 1.063, 1.087, and 1.21 g/ml; the first two density fractions were centrifuged for 24 hours at 40,000 rpm, and the 1.21 fraction for 48 hours at 40,000 rpm. Lipoproteins in the density range 1.063-1.087 consisting of the overlapping LDL, HDL, and HDL₂ (Mahley and Weisgraber, 1974b) were excluded from analysis. LDL and HDL₀ in the density range 1.006-1.063 were separated by Pevikon Block electrophoresis, using the method described by Mahley and Weisgraber (1974b). HDL₀ was obtained in a density range 1.087-1.21. Total cholesterol of plasma and lymph lipoprotein fractions was analyzed by the method of Chiamori and Henry (1959) with minor modifications. Recovery of cholesterol in LDL, HDL, and HDL₂ fractions compared to total plasma and lymph cholesterol was >90% in dogs and >95% in pigs.

**Preparation and Iodination of LDL**

Human LDL with a density between 1.025 and 1.045 were isolated by differential ultracentrifugation of human plasma and labeled with ¹²⁵I as previously described (Angel et al., 1979). After the iodination procedure, ¹²⁵I-LDL was reisolated by centrifugation at density 1.050. On analysis, over 98.5% of the radioactivity was insoluble in 10% TCA. The human ¹²⁵I-LDL co-chromatographed with dog plasma LDL and lymph LDL on agarose electrophoresis.

**Kinetics of Plasma and Lymph LDL**

Under general anesthesia (fluothane), a median sternotomy was performed and a main efferent cardiac lymph trunk was cannulated. One to 200 μCi of ¹²⁵I-LDL were administered iv to each of four dogs weighing 18-35 kg. Cardiac lymph was collected at intervals of 15 minutes during 3 hours. Blood was obtained from the superior vena cava at 2, 3, 5, 10, and 15 minutes and at each subsequent 15 minutes for 3 hours. In an additional experiment, 300 μCi of ¹²⁵I-LDL were injected and, 27 hours later, cardiac lymph and plasma were collected over 5 hours. LDL radioactivity was determined after lipoprotein precipitation with 10% TCA using a Searle γ counter. The log-linear profile of ¹²⁵I-LDL plasma clearance was consistent with a two-pool model (Sniderman et al., 1975). The rapid phase of plasma disappearance (exponential 2), representing equilibration with the extravascular pool, was calculated.
according to the Matthews’ model (Matthews, 1957), using the constants of exponential 1 from the final linear portion of the decay curve (after 2 hours). To measure the specific activity, plasma and lymph LDL were isolated by differential ultracentrifugation (d > 1.006-1.063). The lipoproteins were dialyzed against 0.1% NaCl at pH 7.7. Heparin (40,000 U/ml in 0.15 M NaCl) and 1.06 m MnCl₂ in a ratio of 2,400 U of heparin to 126 mg MnCl₂ (Warnick and Albers, 1978) were mixed and 0.2 ml of the mixture was added to 2 ml of the dialyzed LDL. The sample was mixed on a Vortex mixer and allowed to stand for 15 minutes before centrifugation at 1,000 g for 60 minutes. The supernatant was decanted and the precipitate was washed with a mixture of 0.1% NaCl (2 ml) and heparin-Mn⁺⁺ (0.2 ml) and respun. The supernatant was discarded and an apoprotein precipitate free of excess manganese was obtained by the addition of 1 ml of 5% NaCl and 0.25 ml of 50% TCA at 4°C. After centrifugation at 1,500 g for 30 minutes, the precipitate was sequentially washed and spun four times with 10% TCA-95% ethanol (1:1), 95% ethanol, 95% ethanol-ether (1:1), ether. The precipitate was evaporated to dryness and dissolved in 1 N NaOH and counted. Protein was measured according to Lowry’s method (Lowry et al., 1951) and used to calculate LDL specific activity.

**Results**

**Anatomy of Cardiac Lymphatics**

Both ventricles contained dense regional networks of subepicardial lymph capillaries (Fig. 1) which grouped in relation to the coronary arteries and converged into two larger left and right efferent lymph vessels. These efferent vessels ran separately in the artrioventricular sulcus close to the coronary vessels, then converged toward the root of the aorta and formed a common efferent lymph trunk behind the aorta. Anastomotic interconnections were observed going from right to left, as shown in the schematic illustration (Fig. 1). In 10 of 15 pigs, the common efferent lymph trunk followed the dorsal side of the superior vena cava and drained into a cardiac lymph node between the superior vena cava and trachea. In five of the 15 pigs, the common lymph trunk divided into two or three smaller vessels, one of which passed directly toward the left pretracheal lymph node between the brachiocephalic artery and trachea. At this level, the diameter of lymph channels ranged between 0.3 and 0.6 mm and contained valves that often resisted the retrograde insertion of the catheter. The efferent vessels from the cardiac lymph node followed a cephalad route or emptied into the left pretracheal node.

**Cardiac Lymph Flow**

The cardiac lymph contained no visible red cells. The flow rate varied from 0.9 to 3 ml/hr, with a mean flow rate (± SEM) of 2.0 ± 0.7 ml/hr in four pigs compared to 1.2 ± 0.3 ml/hr in 10 dogs. The observed flow rates of cardiac lymph probably represent the minimal volume of lymph flowing from the heart, as only the largest and most accessible efferent vessel was cannulated. Flow may have been limited further by the effects of anesthesia, surgical manipulation, and changes in heart rate and contractility (Miller et al., 1972; Taira et al., 1976; Michael et al., 1979).

**Lipoprotein Cholesterol Composition**

Agarose electrophoresis of the plasma from pigs and dogs revealed two major bands corresponding to LDL and HDL (Fig. 2). A faint VLDL band was present in the pre-β-region in pig plasma only; it was not detected in dog plasma or in cardiac lymph from either species. Electrophoretograms of dog cardiac lymph showed two major constituents, a faint LDL band and a HDL band; the latter was preceded by a faster α-migrating band (4 in Fig. 2) which was present in dog plasma also. LDL and HDL were visible in pig cardiac lymph, the LDL band being the major lipoprotein detected by Sudan Black B.

The mean concentrations of cholesterol were sig-
CARDIAC LYMPH LIPOPROTEIN
et al
PIG DOG
CARDIAC
LYMPH
PLASMA

FIGURE 2 Agarose electrophoretograms of lipoproteins in 5-μl samples of pig and dog plasma and cardiac lymph applied to the origin 0. The numbered bands correspond to 1-LDL (β), 2-VLDL (pre-β), 3-HDL (α), and 4-a fast α-migrating lipoprotein. The bands were detected with Sudan Black B.

significantly lower in cardiac lymph than in plasma from both species (Table 1). Fractionation of the lipoproteins showed that, in pigs, the cholesterol concentration was considerably lower in cardiac lymph LDL (24.1 ± 9.1 mg/dl) than in plasma LDL (45.7 ± 6.7) and also lower in lymph HDL (19.9 ± 5.7) than in plasma HDL (33.5 ± 5.1) (Table 2) but differences did not reach statistical significance. In dogs, both LDL and HDL cholesterol were significantly lower in cardiac lymph than in plasma (Table 2).

Appearance of 125I-LDL in Dog Cardiac Lymph

After iv injection of 125I-LDL, total plasma radioactivity decreased rapidly during the first 1/2 hour and more slowly thereafter, indicating a multieponential profile consistent with a two-pool model. Figure 3 shows an example of how the plasma clearance curve resolved into two single exponentials. To calculate the constants of the second exponential, i.e., the early portion of plasma clearance which represents equilibration with extravascular pools, the slope (b1) and intercept (C1) were derived (see Methods).

Table 3 shows that the half-time for the 125I-LDL equilibration with the extravascular space (exponential 2) was 0.66 ± 0.16 hour and the half-time of irreversible degradation (exponential 1) was 11.77 ± 1.90 hours. The plasma 125I-LDL appeared in cardiac lymph within 15 minutes (Fig. 3) after injection, rapidly increased in concentration and pl-

Table 1 Cholesterol Concentration in Cardiac Lymph and Plasma

<table>
<thead>
<tr>
<th></th>
<th>Cardiac lymph (mg/dl)</th>
<th>Plasma (mg/dl)</th>
<th>Lymph : plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs</td>
<td>54.9 ± 8.9*</td>
<td>88.8 ± 4.3</td>
<td>0.62</td>
</tr>
<tr>
<td>Dogs</td>
<td>53.6 ± 6.3*</td>
<td>117.4 ± 12.1</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. * P < 0.01 (significance of difference from plasma values)

Table 2 Lipoprotein Cholesterol Concentration in Porcine and Canine Plasma and Cardiac Lymph

<table>
<thead>
<tr>
<th></th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL : HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>45.7 ± 6.7</td>
<td>33.5 ± 5.1</td>
<td>1.37 ± 0.10*</td>
</tr>
<tr>
<td>Cardiac</td>
<td>24.1 ± 9.1</td>
<td>19.9 ± 5.7</td>
<td>1.17 ± 0.22*</td>
</tr>
<tr>
<td>Lymph</td>
<td></td>
<td></td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Dogs</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Plasma</td>
<td>11.6 ± 3.8</td>
<td>76.5 ± 14.5</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Cardiac</td>
<td>4.1 ± 2.3</td>
<td>32.6 ± 7.8</td>
<td>0.13 ± 0.06</td>
</tr>
<tr>
<td>Lymph</td>
<td></td>
<td></td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. NS = not significant.

* The LDL/HDL ratio in pig plasma and cardiac lymph exceeded that of dog (P < 0.001).

† The cholesterol content of the overlapping lipoproteins (d 1.063-1.087) were 9.5 ± 4.5 in dog plasma and 6.2 ± 1.9 in cardiac lymph.
Figure 3  Plasma clearance and cardiac lymph appearance of labeled LDL after iv injection of $^{125}\text{I}}$-LDL in a dog. The half-time was calculated from the formula $t/2 = 0.693/b$ where $b$ was the slope of each exponential.

Table 3  Kinetic Parameters and Half-time for Plasma Clearance and Cardiac Lymph Appearance of $^{125}\text{I}}$-LDL in the Dog

Table 4  Specific Activity of Plasma and Cardiac Lymph following iv Injection of $^{125}\text{I}}$-LDL

Discussion

Whereas the presence of a cardiac lymphatic system in the pig has been known for some time (Johnson and Blake, 1966), a systematic examination of the gross and microscopic anatomy was required to document the distribution and pathway(s) of lymph flow prior to the studies reported here on interstitial lipoprotein composition and kinetics. We have demonstrated lymphatic vessels draining each ventricle and flowing proximally to form right and left efferent lymph vessels which join to form a valved common efferent lymph trunk terminating in a cardiac lymph node. This anatomy is similar to that reported in humans (Feola et al., 1977) and dogs (Julien et al. 1974; Leeds et al., 1979) and it is thus reasonable to assume that it subserves similar physiological functions in the three species.

The data indicate that the LDL:HDL cholesterol ratios are the same in plasma and lymph in individual species but are different between species ($P < 0.001$), cholesterol being mainly in the LDL fraction in pigs and in the HDL fraction in dogs (Table 2). On agarose electrophoresis a faint, faster, $\alpha$-migrating band was seen in canine plasma and a stronger similar band in canine lymph. The ratio of lymph:plasma total cholesterol was 0.62 in pigs and 0.46 in dogs, and on separating the lipoproteins, the LDL and HDL cholesterol concentrations were significantly lower in lymph than in plasma in both species. In pigs, the HDL cholesterol concentration of cardiac lymph was 52% that of plasma, but a large variance precluded statistical differentiation. Our findings differ to some extent from those of Stokke et al. (1974), who found relatively large amounts of VLDL in cardiac lymph, whereas we found very little on electrophoresis (Fig. 2). Furthermore, 90-95% of lymph cholesterol was in HDL and LDL, whereas Stokke et al. (1974) could account for only
48% of the cholesterol in these fractions. Nevertheless, on recalculating their data (Stokke et al., 1974), the LDL:HDL ratios were 0.15 in plasma and 0.18 in cardiac lymph, not very different from our values of 0.14 and 0.13, respectively (Table 2).

The high concentration of LDL in cardiac lymph is of some interest as it is substantially greater than that expected from simple calculations, given the known extravascular distribution of LDL (approximately 20–30% of plasma pool, according to Snederman et al., 1975) and the large interstitial space compared to plasma. Whereas the overall concentration of interstitial LDL is thought to approach 10% that of plasma (Reichl et al., 1977), the cardiac interstitial pool appeared to be enriched as the concentration of LDL cholesterol in cardiac lymph in both pigs and dogs was 35–53% that of plasma (Table 2). The reason for this may be explained in part by the unique large pore structure of cardiac capillary endothelium (Areskog et al., 1964; Karnovsky, 1967; Anversa et al., 1973) through which particles the size of dog LDL (150-300 Å according to Mahley and Weisgraber, 1974b) can readily pass. This mechanism might also explain the rapid appearance (Fig. 3; Table 3) of 125I-LDL in cardiac lymph ($t^{1/2} = 0.08$ hr) compared to exponential 2 ($t^{1/2} = 0.66$ hr) which represents equilibration with the entire extravascular compartment. The role of pulsatile cardiac contractions in the transcapillary flux and net movement of LDL into the extravascular space is also an important consideration, as muscle contraction is known to increase lymph flow.

The possibility that LDL was produced in the cardiac interstitial space by degradation of VLDL is another explanation for the relatively high concentration of LDL in cardiac lymph. This suggestion is deduced from the findings that VLDL is present in cardiac lymph (Julien and Angel, 1978) and that the lymph LDL specific activity did not equilibrate with that of plasma following injection of 125I-LDL (Table 4), indicating continuous addition of unlabeled LDL in the interstitial compartment. The quantitative significance of this latter mechanism remains to be established.

Our findings indicate that cardiac lymph cholesterol is associated primarily with LDL and HDL, that in swine, LDL is the major cholesterol-carrying lipoprotein and that, in dog, HDL is the predominant species in interstitial fluid. The direct entry of plasma LDL into the heart extravascular pool and the high concentration of lipoproteins in cardiac lymph together with the enzymes capable of metabolizing them (Stokke et al., 1974; Julien and Angel, 1980) suggest that the heart interstitial compartment is a potential site of lipoprotein interconversion and lipoprotein-cell interactions. These findings taken together indicate that cardiac muscle cells and connective tissue cells are exposed to very high concentration of lipoproteins, exceeding by orders of magnitude known $K_m$'s of high affinity receptor-mediated binding and internalization of LDL, as described for a variety of mesenchymal cells, e.g., fibroblasts (Reichl et al., 1978), smooth muscle cells (Bierman et al., 1974), and adipocytes (Angel et al., 1979), thus saturating any receptor-controlled lipoprotein uptake mechanism. The significance of these observations with respect to cholesterol flux in the cardiac bed is not known; however, it is worth considering the possibility that the predilection of the coronary vascular bed for atherosclerotic degeneration may be a function of the interstitial lipoprotein concentration and composition as well as the plasma concentration of lipoprotein species.

Acknowledgments

We express sincere appreciation to Laura Sheu and Manuel C. Uy for their expert technical assistance during the course of this work. Dr. Pierre Julien was a Fellow of the Ontario Heart Foundation.

References


Leeds SE, Uhley HH, Meister RB (1979) Application of direct cannulation and injection lymphangiography to the study of the canine cardiac and pulmonary efferent mediastinal lymphatics. Invest Radio 14: 70–78


Lumb GD (1966) Experimentally induced cardiac failure in...
Lipoprotein composition and transport in the pig and dog cardiac lymphatic system.
P Julien, E Downar and A Angel

doi: 10.1161/01.RES.49.1.248

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/49/1/248.citation