ISOLATION AND CHARACTERIZATION OF LIPOPROTEIN-X (LP-X) FROM CANINE

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1. Introduction

In recent years it has been well documented that the characteristic increase in plasma phospholipids and unesterified cholesterol in patients with cholestasis is the result of the presence of an abnormal lipoprotein [1–4]. This abnormal lipoprotein has been designated LP-X. In subsequent studies information was provided with respect to the chemical and physicochemical properties of LP-X and to the structural relations of the proteins to the lipids in this abnormal lipoprotein compound [5–9]. It has also been demonstrated that the presence of LP-X is exclusively limited to patients with cholestasis and obstructive jaundice [10–12]. However, no experimental data are available indicating the site of its formation and the mechanisms regulating its metabolism. In order to investigate this problem in detail it is important to have an adequate animal model available. In this paper we are presenting such a model by the identification of LP-X in canine, after surgical ligation of the common bile duct.

2. Material and methods

2.1. *Animals and surgical treatment*

Four healthy beagle dogs of either sex (age: ranging between 1 and 2 years; body weight: ranging between 6.5 and 10.5 kg) were fed a commercial dog food (Altromen-Trockenfutter; Altrogge, 4937 Lage/Germany) before and after the operation.

For surgical treatment the dogs were anesthetized with Nembutal (Deutsche Abbott GmbH, Ingelheim/Germany) 20 mg/kg body weight. A double ligation of the common bile duct was performed during a 20-min lasting operation. Postoperatively all animals received 1g of Binotal (Bayer AG, Leverkusen) per day.

2.2. *Determination of LP-X*

The test to determine the presence of LP-X in the dogs' serum was performed as previously described using a new technique of precipitating lipoproteins in gels after electrophoresis with polyanionic compounds [13]. For screening purposes the 'LP-X Rapidophor All In' (Immuno AG 1220 Vienna, Industriestrasse 72) was used. In addition whole serum and isolated lipoprotein fractions were analyzed for its electrophoretic behaviour in 1% agarose (Serva, Heidelberg) and 1% agar (Difco–Bacto Agar, Detroit Mich.)-gel electrophoresis employing a solution 0.1 molar in $MgCl_2$ containing 0.25% Na-heparin (Serva, Heidelberg), and 1.3% NaCl. to visualize the lipoproteins after electrophoretic separation [13].

2.3. *Immunochemical methods*

Samples of whole serum, intact and partially delipidized lipoprotein fractions were studied immunochemically using anti-dog antiserum and anti-human albumin antiserum (Behring-Werke, Marburg, Lahn/Germany) as antibodies. Immunoelectrophoresis [14] and double diffusion [15] was performed in 1% agar (Difco–Bacto Agar) employing a barbital buffer, pH 8.7, ionic strength 0.05. The plates were allowed to develop for 24–36 hr at 37°C.

2.4. *Chemical analyses*

Esterified and unesterified cholesterol, triglycerides, phospholipids and protein were determined as described previously [4]. Sodium, potassium, calcium, inorganic phosphorus, glucose, urea, acid, kreatinin, total bilirubin, alkaline phosphatase, SGPT and SGOT were analyzed employing a Technicon Multicanal Autoanalyzer (SMA 12/60; Technicon Instruments Corp., Tarrytown, N.Y.) equipped with the standard requirements.

2.5. *Isolation and partial delipidization of LP-X*

LP-X was isolated from dog and human serum after its presence was demonstrated by the LP-X Rapidophor test. Blood samples were collected, allowed to clot and the serum was recoverd by low speed centrifugation. LP-X was isolated by a previously described procedure, which combines heparin precipitation, ethanol precipitation and ultracentrifugation [4]. Partial delipidization of purified dog LP-X fractions was accomplished according to the procedure by Gustavson [16].

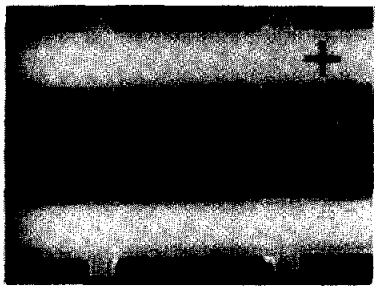

Fig. 1. LP-X pattern obtained with the 'Rapidophor All In' set for the determination of LP-X in whole serum. c = LP-X positive human serum for control; 1 = dog serum before ligation of the bile duct; 2 = dog serum 8 hr after ligation of the bile duct; 3 = dog serum 16 hr after ligation of the bile duct; 4 = dog serum 24 hr after ligation of the bile duct; 5 = dog serum 38 hr after ligation of the bile duct; 6 = dog serum 72 hr after ligation of the bile duct.

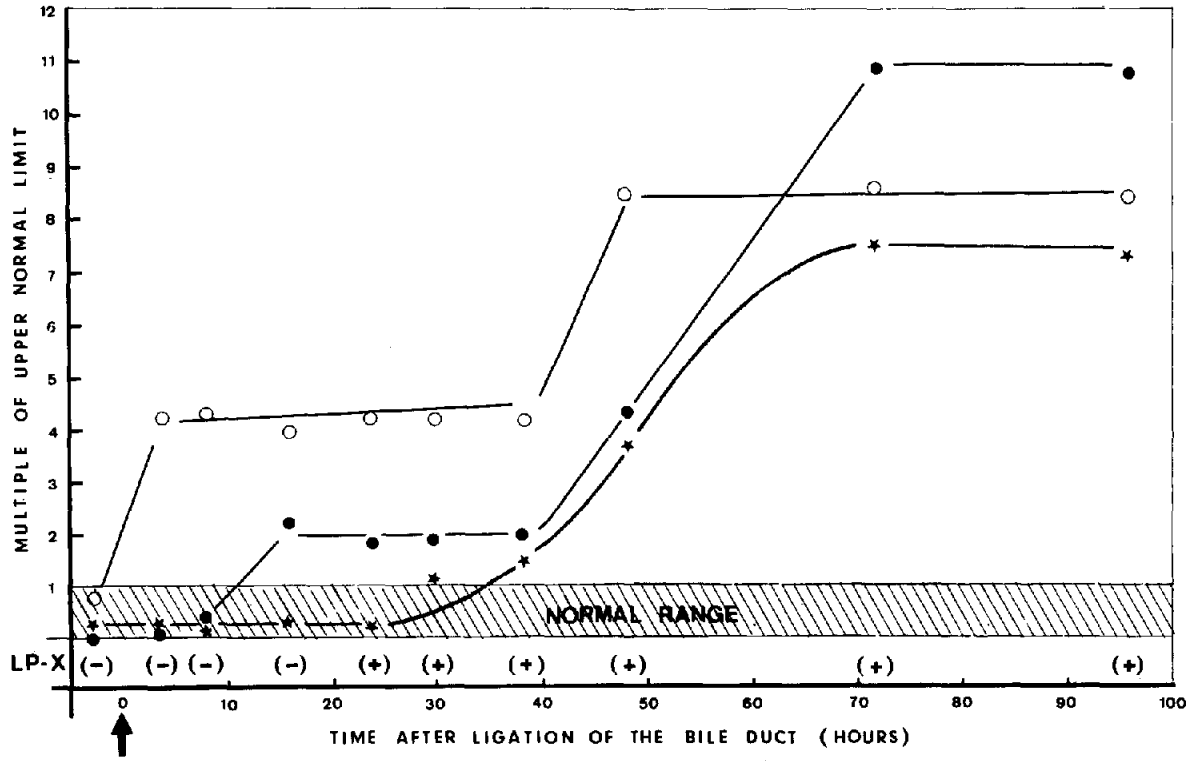

Fig. 2. Alkaline phosphatase (●-●-●) and alanine aminotransferase (SGPT) (o-o-o) activity, total bilirubin (*-*-*) concentration and result of the LP-X test in canine during a follow-up study after ligation of the common bile duct. ↑ Ligation of the bille duct.

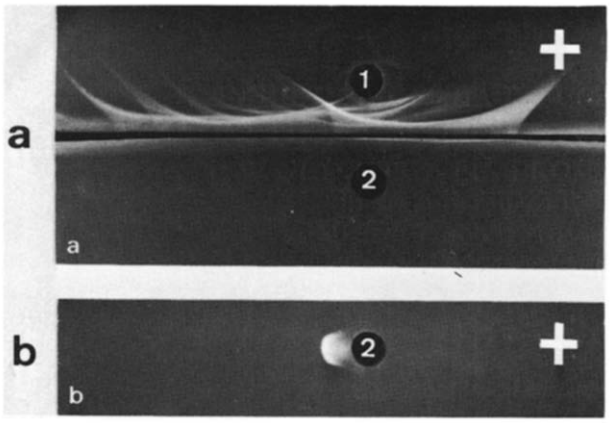

Fig. 3. Agar-electrophoresis pattern of canine control serum (1) and isolated LP-X from dog (2); a = Immunoelectrophoresis technique using anti canineserum; b = polyanion precipitation technique (0.1 M $MgCl_2$ 0.25% Na-heparin and 1.3% NaCl).

2.6. *Electronmicroscopy of isolated dog LP-X fraction*

Electronmicroscopy of isolated dog LP-X fraction was kindly conducted by Dr. Kern, Anatomisches Institut, University of Heidelberg, using previously described methods [9].

3. Results

In canines it is possible to induce the formation of lipoprotein-X after surgical ligation of the common bile ducts. A follw-up study of four animals demonstrated that this abnormal plasma lipoprotein occurs 24–30 hr after ligation of the bile duct. From then on the plasma concentration of LP-X increases rapidly with the duration of cholestasis (see figs. 1 and 2). While sodium, potassium, calcium, inorganic phosphosus, glucose, urea, uric acid, kreatinin remained normal during a postoperative period of four days, both aminotransferases (SGOT, SGPT) total bilirubin and alkaline phosphatase showed a marked increase after the operation (see fig. 2).

Chemical and physicochemical fractionaltion of canine serum indicated an identical behaviour of canine lipoprotein-X in comparison to lipoprotein-X from humans [4,6]. LP-X from canines was found to be a low density lipoprotein within the density fraction d 1.006–1.063 g/ml. As LP-X from humans, dog LP-X is present in the Cohn-Fraction IV–VI [4] and precipitates with Na-heparin and $MgCl_2$. It can be

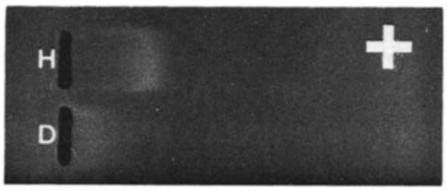

Fig. 4. Agarose-electrophoresis pattern: H = isolated LP-X from humans; D = isolated LP-X from canine.

isolated by a combination of ultracentrifugation, heparin-precipitation and Cohn-fractionation [4], a procedure preserving the native structure and properties of the particle.

Electron micrographs of isolated canine LP-X revealed spherical particles with a diameter ranging from 300–700 Å, a strong tendency to aggregate and to form rolls, identical to those previously described for human LP-X [7,9].

The protein lipid composition of canine LP-X shows the same unique characteristics as human LP-X, consisting of 5.4% protein, 50% phospholipids, 28% cholesterol of which only trace amounts are esterified, and 8% triglycerides. It migrates on agar electrophoresis (see figs. 1 and 3) towards the cathode and on agarose electrophoresis towards the anode with a mobility slightly less than that of human LP-X (see fig. 4).

Purified LP-X from dog does not react immunochemically with anti-canine serum. However, after partial delipidization the same preparation gave a positive reaction not only with anti-canine antiserum,

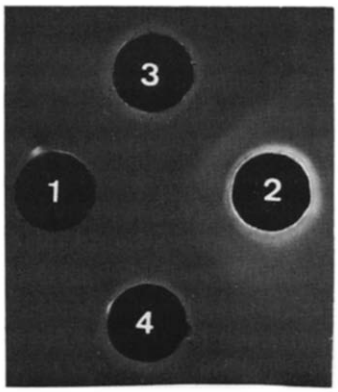

Fig. 5. Immunodiffusion pattern of purified intact LP-X from dog (1), (2) after partial delipidization against anti-human albumin antiserum (3) and anti-canine-antiserum (4).

but also with antiserum to human albumin (see fig. 5.). This phenomenon has already been described for isolated LP-X from humans, indicating that albumin plays an important role for maintaining the structure of LP-X. Furthermore, this experiment demonstrates common antigenic determinants of human and dog albumin.

4. Discussion

Results of the present study indicate that the abnormal low density lipoprotein (LP-X) characteristic for cholestasis in humans can also be found and isolated in canines after surgical ligation of the common bile duct. The structure as well as the unique physicochemical and chemical properties of human- and canine-LP-X are identical. Thus, this animal model seems adequate for the study of LP-X metabolism. Of particular interest in this respect is the fact, that LP-X may now easily be identified in whole serum without a specific antiserum using a precipitation technique with polyanionic compounds after electrophoresis on agar gel [13, 17].

Acknowledgement

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References

[1] Russ, E.M., Raymunt, J. and Barr, D.P. (1956) J. Clin. Invest. 35, 133.
[2] Switzer, S. (1967) J. Clin. Invest. 46, 1855.
[3] Burstein, M. and Caroli, J. (1967) Rev. Franc. Etudes Clin. Biol. 12, 898.
[4] Seidel, D., Alaupovic, P. and Furman, R.H. (1969) J. Clin. Invest. 48, 1211.
[5] Mills, G.L., Seidel, D. and Alaupovic, P. (1969) Clin. Chim. Acta 20, 239.
[6] Seidel, D., Alaupovic, P., Furman, R.H. and McConathy, W.J. (1970) J. Clin. Invest. 49, 2396.
[7] Hamilton, R.L., Havel, R.J., Kane, J.P., Blaurock, A.E. and Sata, T. (1971) 172, 475.
[8] Picard, J., Veissiere, D., Voyer, F. and Bereziat, G. (1972) Clin. Chim. Acta 36, 247.
[9] Seidel, D., Agostini, B. and Muller, P. (1972) Biochim, Biophys. Acta 260, 146.
[10] Seidel, D., Gretz, H. and Ruppert, C. (1973) Clin. Chemistry 19, 86.
[11] Ritland, S., Blomhoff, J.P., Elgjo, K. and Gjone, E. (1973) Scand. J. Gastroenterol. 8, 155.
[12] Poley, J.R., Alaupovic, P., McConathy, W.J., Seidel, D. Roy, C.C. and Weber, A. (1973) J. Lab. Clin. Med. 81, 325.
[13] Seidel, D., Wieland, H. and Ruppert, C. (1973) Clin. Chemistry 19, 737.
[14] Grabar, P. and Williams, C.A. (1955) Biochim. Biophys. Acta 17, 67.
[15] Ouchterlony, Ö. (1953) Acta Pathol. Microbiol. Scand 32, 231.
[16] Gustafson, A. (1965) J. Lipid. Res. 6, 512.
[17] Wieland, H. and Seidel, D. (1973) Eut. Med. Wochschr. 98, 1474.