APOPROTEIN A-I, A-II, AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN DIABETES MELLITUS

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The serum levels of apoprotein A-I (A-I), apoprotein A-II (A-II) and high density lipoprotein-chlesterol (HDL-C) were measured in 144 diabetic patients (almost non-insulin-dependent, mean age 59.3) and 35 normal subjects as control (mean age 43.8). The A-I and A-II were determined by single radial immuno-diffusion using specific anti-A-I and anti-A-II serum (Daiichi Kagaku Co., Japan). HDL-C was determined by phsopho-tungstate-Mg^{++} method.

Diabetic patients were devided 4 groups as follows : no hyperlipidemia 56 cases, hypertriglyceridemia (>130 mg/dl) without hypercholesterolemia 45 cases, hyper-cholesterolemia (>250 mg/dl) with or without hypertri-glyceridemia 16 cases and presence of extra pre-beta band on lipoprotein electrophoretic pattern 11 cases.

In the control, The A-I, A-II and HDL-C levels were 135.6 ± 22.4 mg/dl (mean ± SD), 32.1 ± 5.1 mg/dl, 52.5± 15.7 mg/dl respectively. In the diabetic patients, A-II levels of hyper-cholesterolemia group (36.4 ± 5.9 mg/dl) were significantly higher than hypertriglyceridemia group (29.0 ± 6.0 mg/dl) ($p < 0.025$). HDL-C level of hypertriglyceridemia group (35.7 ± 5.9 mg/dl) was lower than control group ($P < 0.025$). There were no significant difference in normolipemic and presence of extra pre-beta band groups.

It was suggested that high A-II is related to increased serum cholesterol, low HDL-C is related to increased serum triglyceride. Further studies is necessary to confirm the relationship between these results.

Memo:

PURIFICATION AND PROPERTIES OF APOLIPOPROTEIN A-I AND A-II FROM HUMAN SERUM HIGH DENSITY LIPOPROTEINS (HDL)

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HDL has attracted increasing interest in recent years following the finding of an inverse correlation between coronary heart disease and serum HDL level. The protein moiety of HDL contains two major polypeptides, apoA-I and apoA-II, and several minor components. Precise quantitation of the apoproteins is required in order to assume their role in structure and function of HDL as well as its abnormality. We undertook the purification of ApoA-I and ApoA-II from human serum HDL and immunization of these purified proteins. Monospecific antiserum reacting with apoA-I and apoA-II were obtained. Methods : HDL (density 1.063-1.21) was isolated from fasting plasma of normolipidemic donors by sequential ultracentrifugation and fractionated on Sepharose 6B column chromatography, and delipidated with ether-methanol under -20°C. The delipidated preparation was chromatographed on DEAE Sepharose CL-6B, equilibrated with 0.03M Tris/HCl, pH8.0 containing 6M urea, and was then eluted with a linear gradient of NaCl from 0 to 0.125M NaCl to yield crude apoA-I and A-II fraction. Crude ApoA-I and ApoA-II fractions were further purified by gel filtration on SephadexG-100(A-I) or SephadexG-75(A-II) equilibrated with 0.1M Tris/HCl pH8.6 containing 6M urea. The purity of these purified proteins was check by DISC-PAGE containing 6M urea SDS-PAGE, various immuno chemical methods and amino acid analysis. New Zealand white rabbit were immunized with either purified ApoA-I or ApoA-II mixed with complete Freunds adjuvant and injected into multiple intradermal sites at every one week. Rabbits with significant titers at 8 weeks were bled 2 weeks later. Antiserum specificity was checked with double immunodiffusion methods and immuno electrophoresis.

Memo:

THE DENATURATION BY LINOLEIC ACID HYDROPEROXIDE AND AN AUTO - DENATURATION IN HUMAN LIPOPROTEINS

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A Low Density Lipoprotein (LDL) is a carrier of lipid peroxide, and a hydroperoxide selectivelly or preferentially denatures LDL or β-lipoproteins are well known. Here we report that Very Low Density Lipoprotein (VLDL) and High Density Lipoprotein (HDL) are also denaturated by the interaction with linoleic acid hydroperoxide. Additionally, the study touchs on an auto-denaturation of each lipoproteins.

Human serum was interacted with several concentration of linoleic acid hydroperoxide at 37°C for 8 hours. Lipid staining pattern and Schlieren pattern of the interacted lipoproteins were analysed using 3.5% polyacrylamide gel-disc electrophoresis and analytical ultracentrifuge. A LDL was easily denaturated by the lower concentration of linoleic acid hydroperoxide, and the heterogeneous LDL were formed on the lipid staining pattern and Schlieren pattern. Both lipoproteins VLDL and HDL_2 were also denaturated by the interaction with linoleic acid hydroperoxide. However, the effective concentration of linoleic acid hydroperoxide was significantly higher than that of LDL.

An auto-denaturation of isolated lipoprotein was occured by the contact with a large quantity of oxygen at 37°C. Especially, the heterogeneous standard was dependent on the concentration of cholesterol ester in each lipoprotein,i.e., a cholesterol-rich VLDL, LDL, and HDL_2 were markedly denaturated in condition with a large quantity of oxygen. The study demonstrates the lipid staining pattern and Schlieren pattern of denaturated lipoprotein , and discusses their characterization.

Memo:

STUDIES ON IgM-K/IgG MIXED CRYOGLOBULIN

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Seventy-two cases(90%) out of 80 cases of cryoglobulinemia in our series were mixed cryoglobulins.[1] If the immunological reactions are concerned itself with the formation of cryoprecipitate, one of the cryoglobulin components must have the antibody activity against the others.

This report describes studies on the IgM/IgG mixed cryoglobulin from the standpoint of immun-complex. Each component of the cryoglobulin was purified by the means of gel filtration column chromatography under the acidic pH condition. Enzymatic digestion studies of the IgM M-protein and polyclonal IgG showed that the M-protein had the antibody like activity against IgG Fc portion. The denaturation studies on polyclonal IgG using enzymes such as hyaluronidase, heat and some chemical reagents suggested that the antigenic determinant structure on the IgG molecule may play very important role on the temperature dependent precipitation reaction.

1) S. Hashimoto. The Physico-Chemical Biology. 24 (4), 289-293, 1981.

Memo:

MONOCLONAL GAMMOPATHY ASSOCIATED WITH ANTIBODY ACTIVITY TO HETEROLOGOUS ALPHA-2 MACROGLOBULINS

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Second case of monoclonal gammopathy associated with antibody activity to heterologous alpha-2macroglobulin is presented in this report.

The patient was 63 years old male, who was admitted to Nakatsu Hospital with the complaint of abdominal pain, who has detected to have approximately 9.8 % of monoclonal peak (M-P) at slow gamma area by cellulose acetate electrophoresis. But immunoelectrophoresis of the serum failed to define the type of M-P, because all of the monospecific antisera to human IgG, IgA, IgM, IgD, IgE and light chains, raised in rabbit, were reacted with the M-P, when unpurified antisera were used. Although there were no such peculier precipitation lines were detected when purified monospecific antisera were used. The peculier precipitation line, identical to that was produced with unpurified antisera, were detected when normal rabbit or horse serum was added to the antibody trench, instead of those monospecific antisera to human immunoglobulins. Therefore, we speculated that the M-P has an antibody activity to heterologous proteins. Heterologous protein, reacted with the M-P was noticed to have an electrophoretic mobility of alpha-2 fraction in agar gel electrophoresis and also speculated to have high molecular weight by column chromatographic analysis.

Similar case had been reported by Seligman et al. in which case the M-P was confirmed to react specifically to rabbit, horse and pig alpha-2 macroglobulin.

Memo:

RAPID PURIFICATION OF MITOCHONDRIA DNA USING AGAROSE GEL ELECTROPHORESIS

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The maternal inheritance of human mitochondrial DNA was demonstrated by Case and Wallance (1981) using restriction endnuclease cleavage pattern of the DNA. Normally, DNA fragments after agarose gel electrophoresis are identified by Southern gel hybridization procedure or ethidium bromide staining method.

The simple and rapid method to purify DNA from human placenta mitochondria was improved using agarose gel electrophoresis. The original methods described by Yang and Wu (1979) was modified. The slice of the agarose gel containing DNA isofragment after electrophoresis is dissolved in 4 volumes of 6 M $NaClO_4$ at 50 - 60°C for few min. DNA is stained by ethidium bromide. The DNA in the dissolved agarose adheres to a single 10 mm-diamiter glass filter (Whatman GF/C) which are placed on a single-glass filtration set under water aspiration. After that, the glass filter is washed with 1 ml of $NaClO_4$-Tris buffer and then 1 ml of isopropanol and 1 ml of ethanol and then, dried in the room temparature for few min using hair dryer. The DNA on the glass filter is eluted by reaction buffer of restriction endonuclease. The elution buffer of 100 µl is added to the filter containing DNA in the Eppendrof microtube. The bottom of tube is piered with needle and then eluate is collected by 12000 g for 10 sec. When 30 g of placenta is used, Ca. 10 µg of purified DNA was obtained in the present method. ECo-RI, Hind-III and Hha-I were used in the present study as restriction endnuclease.

Memo: