ERYTHROCYTE OSMOTIC FRAGILITY IN VARIOUS LIVER DISEASES—APPLICATION OF COIL PLANET CENTRIFUGE SYSTEM

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Summary

A change in erythrocyte osmotic fragility was observed in various liver diseases by means of the coil planet centrifuge (CPC) system, and the relationship between changes in it and in serum lipids was studied. According to the CPC classification of hemolytic patterns of L, M, T and R, the frequency of appearance of T and R increased in liver cirrhosis and primary hepatoma. Hemolytic start and end points both changed considerably in primary hepatoma, acute hepatitis and liver cirrhosis. Change of hemolytic end point which shifted to the hypotonic side is more prominent than that of hemolytic start point. The hemolytic end point showed an inverse correlation to serum alkaline phosphatase and LAP, and correlation to pseudocholinesterase and albumin. Among the relations of red cell fragility and lipids of the lipoprotein fractions, free cholesterol and the ratio of free cholesterol to phospholipid in high density lipoprotein were both in remarkable inverse correlation to the hemolytic end point. Free cholesterol in high density lipoprotein was concluded one of the most important determinants of erythrocyte osmotic fragility.

Key Words: coil planet centrifuge system, liver diseases, erythrocyte osmotic fragility, lipoprotein, serum cholesterol.

Introduction

It is well known that erythrocyte membrane lipid component increases and osmotic fragility decreases in liver diseases1-7). Erythrocyte osmotic fragility has been measured so far by Papa's method, using a dilution series of saline solution, but it has many inconvenient points8). The coil planet centrifuge system used in this study is a method9,10) to measure the hemolytic pattern. A very small amount of blood that is injected into the coil-enclosing solutions of a consecutive concentration gradient is moved by centrifugation from the hypertonic to the hypotonic side and hemolytic patterns in the coil are measured densitometrically. It is recognized to be useful in Japan in the diagnosis of hemolytic diseases. Kitashima studied the results obtained by CPC in liver diseases and concluded that CPC could be used for the diagnosis of liver diseases11,12). To investigate factors determining erythrocyte fragility in hepatobiliary diseases, erythrocyte
osmotic fragility was measured using CPC in various liver diseases; serum lipoproteins were fractionated by ultracentrifugation; cholesterol, triglyceride, and phospholipid were measured in each lipoprotein fraction, and their relations to the results of CPC were studied.

Materials and Methods

Normal control group: This group consisted of 28 persons (10 males and 18 females) without any particular disease in anamnesis and with no abnormality in fasting blood sugar, serum total cholesterol, triglyceride, total protein, albumin (ALB), total bilirubin (TB), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase, alkaline phosphatase (ALP), lactic dehydrogenase, urea nitrogen, creatinine, uric acid and serum electrolytes, with an average age of 33 years old. Disease group: Eleven cases of acute hepatitis (AH), 28 cases of chronic hepatitis (CH), eight cases of alcoholic liver injury (ALI), eight cases of fatty liver (FL), 26 cases of liver cirrhosis (LC), of which eight cases were decompensated, and eight cases of primary hepatoma with liver cirrhosis (PH), 117 cases in total were studied. All cases of AH, 22 cases of CH, three cases of FL and 17 cases of LC were diagnosed histologically, and PH was confirmed by autopsy in all cases. The other cases were diagnosed clinically.

Method of measuring erythrocyte osmotic fragility: Hemolytic pattern was measured using coil planet centrifuge apparatus (Biomedical Systems Corp. Tokyo), in accordance with the method of Shibata et al. Ten μl of blood containing 20 μg of EDTA was injected into the plastic coil with an inside diameter of 0.3 mm and length of 3 m with a linear osmotic gradient from 150 to 30 mOsm of NaCl, and then both ends were closed. The blood-containing coil was incubated at 37°C for 10 min, and centrifuged perpendicularly in a acceleration field at 1,600 rpm for 10 min after the coil was fixed in a slowly rotating coil holder. The hemolytic band of the coil was classified into four hemolytic patterns, L, M, T and R, with a densitograph (Fig. 1). The hemolytic peak on the side of high osmotic pressure was named the L type, that in the center the M type, that over the sides of high and low osmotic pressures showing a trapezoid form the T type, and that on the side of low osmotic pressure, the R type. The hemolytic starting point (HSP) and hemolytic end point (HEP) were also obtained.

Methods of routine liver function tests: Determinations of ALB, TB, GOT and ALP were performed by the standard auto-analyzer methods (Sequential Multiple Analyzer with a Computer, Technicon Instruments Corp., N.Y.). Pseudocholinesterase (CHE) was measured by the thiocholine-DTN B method. LCAT activity was measured by the enzymatic method.

Method of analyzing lipoproteins: Sera of four cases of LC, two cases of PH and one case of FL after fasting more than 12 hours were