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ISOLATED PARENCHYMAL, KUPFFER AND ENDOTHELIAL RAT LIVER CELLS CHARACTERIZED BY THEIR LYSOSOMAL ENZYME CONTENT

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SUMMARY

The activities of nine key lysosomal enzymes were determined in isolated parenchymal cells and in isolated Kupffer and endothelial cells purified by centrifugal elutriation. Compared with parenchymal cells, all specific lysosomal enzyme activities were much higher in Kupffer and endothelial cells. Kupffer cells showed a specific enrichment in acid lipase, β-glucuronidase, cathepsin D and aminopeptidase B, whereas the specific activities of acid phosphatase, acid DNAse, β-acetylglucosaminidase and arylsulphatase B were much higher in endothelial cells as compared with Kupffer cells; β-galactosidase was equally present in both cell classes. The distribution of lysosomal enzyme activities reflects the role of Kupffer and endothelial cells in the clearance and degradation of serum lipoproteins and glycoproteins.

INTRODUCTION

About two-thirds of the total cell number in the mammalian liver consists of parenchymal cells and about one third of cells which line the blood sinusoids. Kupffer and endothelial cells represent the great majority of these sinusoidal cells (1). This cell population plays an important role in the clearance of abnormal or foreign substances and particulate material, including senescent erythrocytes, liposomes, microorganisms and endotoxin (2). The sinusoidal cells also have a specific function in the removal of cholesterol esters (3) and glycoproteins (4,5) from the circulation. In view of these important tasks, it has been of interest to distinguish between Kupffer and endothelial cells with respect to their involvement in the sinusoidal cell functions. Morphological and cytochemical observations during clearance studies in vivo indicated a different reaction mechanism to foreign material in the two cell types (6), but this experimental approach generally suffers from a lack of quantitative data.

We have used a different approach to assess the contributions of Kupffer and endothelial cells to the clearance functions of the sinusoidal cells. The

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extracellular material endocytosed by the cells is exposed to the lysosomal digestion system. The development of a technique for the preparation of highly purified Kupffer and endothelial cell suspensions from the rat liver (1) enabled us to determine the qualitative and quantitative distribution of a number of enzymes involved in the main lysosomal functions in both sinusoidal cell classes and to make a comparison with the enzymes in isolated parenchymal cells. The results reflect the capacity of Kupffer and endothelial cells for the hydrolysis of specific substrates and thus give insight into the functional differences of both cell types.

MATERIALS AND METHODS

Preparation of parenchymal, Kupffer and endothelial liver cells. Parenchymal and sinusoidal cells were isolated from livers of 3-month-old female BN/BiRij rats (140-170 g): parenchymal cells by collagenase treatment (7) and sinusoidal cells by pronase treatment (8). Erythrocytes and cell debris in the sinusoidal cell suspensions were removed by centrifugation in a Metrizamide (Nyegaard and Co.) solution with a density of 1.089 g/cm^3 at 21^{o}C (8). Kupffer and endothelial cells were separated by centrifugal elutriation, as described previously (1,8). At a constant rotor speed of 2500 rpm, three subsequent fractions were collected: lymphocytes, endothelial cells and Kupffer cells at flow rates of 13.5, 23.0 and 40.0 ml.min^{-1}, respectively. The cells were identified cytochemically (9) and by their electronmicroscopic morphology (9). Cell counts were performed with a hemocytometer. Cell viability was estimated from the percentage of cells which excluded 0.25% trypan blue.

Measurement of lysosomal enzyme activities. The activities of 9 lysosomal enzymes were determined after preincubation of freshly purified cell suspensions with 0.05% Triton X-100 for 20 min at 0^{o}C. Acid phosphatase (EC 3.1.3.2) activity was determined at pH 5.0 with 4-methylumbelliferyl dihydrogen phosphate (10) as the substrate. The activity of cathepsin D (EC 3.4.23.5) was estimated from the amount of tryptophan liberated from bovine hemoglobin at pH 3.6 (1). The activities of β-galactosidase (EC 3.2.1.23), acid lipase (EC 3.1.1.3) and β-acetylglucosaminidase (EC 3.2.1.30) were determined with the 4-methylumbelliferyl derivates of 5.8 mM β-D-galactopyranoside in 0.08 M acetate buffer pH 3.6, 1.5 mM oleate in 0.08 M citrate-phosphate buffer pH 4.4 and 6.7 mM 2-acetamido-2-deoxy-β-D-glucopyranoside in 0.04 M citrate buffer pH 3.7, respectively. The amount of 4-methylumbelliferone was determined fluorimetrically (excitation 365 nm, fluorescence 448 nm) in 0.5 M glycine-NaOH buffer pH 10.7.

Aminopeptidase B (EC 3.4.12A.1) activity was determined with 0.6 mM arginine-β-naphthylamide in 0.01 M acetic acid, adjusted to pH 5.6 with 0.1 N NaOH. The amount of β-naphthylamine was estimated fluorimetrically (excitation 340 nm, fluorescence 413 nm) in 0.5 M glycine-NaOH buffer pH 10.3.

After incubation with 1.0 mM phenolphthalein-β-D-glucuronic acid in 0.08 M acetate buffer, pH 5.0, the activity of β-glucuronidase (EC 3.2.1.31) could be calculated from the amount of phenolphthalein measured spectrophotometrically at 540 nm in 0.5 M glycine-NaOH buffer, pH 10.7.

The activity of arylsulphatase B (EC 3.1.6.1) was determined with 30 mM nitrocatechol sulphate in 0.13 M acetate buffer pH 5.7. The enzyme reaction was terminated with 1.7% phosphotungstic acid. After centrifugation, the supernatant was mixed with an alkaline quinol reagent. The amount of nitrocatechol was measured spectrophotometrically at 510 nm.

The activity of acid DNAse (EC 3.1.4.6) was determined with 0.8 $mg.ml^{-1}$ calf thymus DNA in 0.17 M acetate buffer pH 5.0 with 0.17 M KCl. The incubation was terminated with 9.5% perchloric acid. After centrifugation, the amount of nucleotides in the supernatant was determined spectrophotometrically at 260 nm.

For the measurement of cellular DNA, the cell suspension was mixed with 5.1 µM diamidino-2-phenylindole.2 HCl in 0.6 M NaCl and 5 mM HEPES pH 7.5. The amount of DNA was calculated from the increase in fluorescence at 449 nm, at excitation 358 nm, compared to a blank without cell suspension. Calf thymus DNA was used as the standard.

RESULTS

Preparations of parenchymal, endothelial and Kupffer cells which were highly purified and which consisted almost totally of viable cells were obtained (Table I). Since about 10×10^7 parenchymal cells, 42×10^6 endothelial cells and 12×10^6 Kupffer cells are present per g liver in a 3-month-old rat (1,9), the yield of purified endothelial and Kupffer cells corresponded to a recovery of about 60% and that of parenchymal cells to one of about 25%. Isolated Kupffer cells contain almost three times the amount of protein as is found in

Table I. General characteristics of purified parenchymal, endothelial and Kupffer cells isolated from rat liver

	Parenchymal cells	Endothelial cells	Kupffer cells
Yield (10^6 cells/g liver)	25.3 ± 2.6 (8)	22.5 ± 1.3 (5)	7.5 ± 0.7 (8)
Purity (%)	98 ± 2 (8)	96 ± 2 (5)	83 ± 4 (8)
Viability (%)	91 ± 2 (8)	96 ± 1 (5)	92 ± 1 (8)
Protein content (µg/10^6 cells)	1280 ± 100 (4)	46 ± 3 (5)	114 ± 4 (8)
DNA content (µg/10^6 cells)	39.5 ± 1.9 (4)	7.2 ± 0.6 (5)	5.5 ± 0.9 (4)

Means ± S.E.M., number of different cell preparations in parentheses.

Table II. Lysosomal enzyme activities in parenchymal, endothelial and Kupffer cells, expressed per 10^6 cells

	Parenchymal cells (n = 4)	Endothelial cells (n = 5)	Kupffer cells (n = 8)
Acid phosphatase	46.3 ± 3.2	7.5 ± 0.6	11.8 ± 1.2
Acid lipase	19.0 ± 3.6	1.3 ± 0.1	6.2 ± 0.4
Acid DNAse	23.3 ± 5.7	17.3 ± 1.8	26.1 ± 5.1
Cathepsin D	0.7 ± 0.0	0.3 ± 0.0	2.0 ± 0.2
Aminopeptidase B	9.9 ± 1.3	0.4 ± 0.1	1.4 ± 0.2
β-Galactosidase	3.2 ± 0.2	1.1 ± 0.0	2.4 ± 0.2
β-Glucuronidase	8.2 ± 0.6	0.9 ± 0.0	3.2 ± 0.2
β-Acetylglucosaminidase	88.2 ± 6.8	13.6 ± 0.9	16.4 ± 0.8
Arylsulphatase B	12.4 ± 1.1	7.1 ± 1.0	4.8 ± 0.7

Enzyme activities are expressed as nmoles 4-methylumbelliferone (acid phosphatase, acid lipase, β-galactosidase, β-acetylglucosaminidase), nmoles nucleotide equivalents (acid DNAse), nmoles tryptophan (cathepsin D), nmoles β-naphthylamine (aminopeptidase B), nmoles phenolphthalein (β-glucuronidase) or nmoles nitrocatechol (arylsulphatase B) released per min at 37°C. Values are the means ± S.E.M.; n is the number of different cell preparations.

endothelial cells. Concerning the data for parenchymal cells, it should be mentioned that, in a 3-month-old rat, mononuclear and binuclear diploid, tetraploid and octaploid parenchymal cells are present, resulting in a great cellular heterogeneity within a parenchymal cell suspension.

Several lysosomal enzymes, selected for their role in the hydrolysis of a variety of substrates were assayed in the purified cell preparations. The enzyme activities found were expressed on both a cellular (Table II) and a protein basis (Table III). Due to their much higher protein content, parenchymal cells show per individual cell the highest activity of all enzymes. In contrast, all specific activities are higher in endothelial and Kupffer cells than in parenchymal cells. For most enzymes a selective enrichment was observed for either endothelial cells or Kupffer cells, as judged from the ratio of the specific activities for endothelial cells to those for Kupffer cells (Table III).

Table III. Lysosomal enzyme activities in parenchymal, endothelial and Kupffer cells isolated from rat liver, expressed per mg protein

	Parenchymal cells (n=4)	Endothelial cells (n=5)	Kupffer cells (n=8)	Ratio Endothelial/ Kupffer cells
Acid phosphatase	36.3 ± 2.1	165.3 ± 21.6	104.6 ± 10.3	1.6
Acid lipase	12.4 ± 2.0	27.1 ± 1.5	53.1 ± 5.3	0.5
Acid DNAse	13.9 ± 3.6	376.9 ± 40.8	235.6 ± 43.4	1.6
Cathepsin D	0.5 ± 0.0	6.4 ± 0.4	17.4 ± 1.8	0.4
Aminopeptidase B	7.7 ± 1.0	9.2 ± 1.2	12.5 ± 1.4	0.7
β-Galactosidase	2.5 ± 0.1	24.1 ± 1.2	21.0 ± 1.6	1.1
β-Glucuronidase	6.4 ± 0.4	19.6 ± 1.2	28.4 ± 2.1	0.7
β-Acetylglucos-aminidase	68.8 ± 3.3	299.7 ± 33.0	144.3 ± 6.6	2.1
Arylsulphatase B	9.7 ± 0.7	149.4 ± 18.0	42.7 ± 7.0	3.5

Enzyme activities are expressed as indicated in Table II.

The specific activities of acid phosphatase, acid DNAse, β-acetylglucosaminidase and arylsulphatase B are much higher in endothelial cells, whereas acid lipase, cathepsin D, aminopeptidase B and β-glucuronidase show higher activities in Kupffer cells.

In separate control experiments, it was shown that the activities of lysosomal enzymes in the various liver cell types had not undergone changes during the isolation procedure (c.f. ref. 8 and 11).

DISCUSSION

The lysosomal apparatus in Kupffer and endothelial cells is well developed. Morphometric studies revealed that 13.6% of the volume of Kupffer cells and 6.9% of that of endothelial cells were made up of lysosomal structures and that Kupffer cells contributed 26% and endothelial cells 17% to the total volume of lysosomes in the rat liver (12). The important volumetric contribution of

the lysosomal structures in Kupffer and endothelial cells can be related to their functional capacity, since isolated sinusoidal liver cells show relatively high specific lysosomal enzyme activities as compared with the activities in isolated hepatocytes (13-15). Although isolated sinusoidal cell preparations are often referred to as Kupffer cells, they actually consist mainly of two cell types, viz., Kupffer cells and endothelial cells, with about 2-3 times more endothelial cells than Kupffer cells (8). Consequently, it was not known to what extent the lysosomal enzymes demonstrated for the sinusoidal cell suspensions were present in Kupffer or endothelial cells. The results of Tables II and III now show that both cell types contain a complete set of all lysosomal enzymes investigated so far; this enables the catabolism of a variety of compounds.

It is of interest to determine whether the data obtained on the distribution of lysosomal enzymes in endothelial and Kupffer cells may confirm functions asigned to one of these cell types on the basis of other experimental evidence. Sinusoidal cells play a quantitatively important role in the uptake and degradation or circulating lipoproteins. Preparations of these cells take up cholesterol moieties of serum lipoproteins (3) and are enriched in acid lipase (15), which is capable of hydrolysing cholesterol esters and triglycerides (16). The localisation of the heparin-releasable hepatic lipase on the surface of the endothelial cells - an enzyme which may mediate the uptake of cholesterol from lipoproteins (17) - is suggestive for a preferential degradation of lipoproteins in endothelial cells. Our results, however, show that Kupffer cells contain more acid lipase on both a cellular and protein basis as compared with endothelial cells. This observation indicates a role for Kupffer cells also in lipoprotein catabolism.

Besides the uptake of lipoproteins, the sinusoidal cells are involved in the selective clearance of circulating glycoproteins, lipopolysaccharides and mucopolysaccharides. Endothelial cells are 2-6 times more active than Kupffer cells in the internalization of mannose- or N-acetylglucosamine-terminated

glycoproteins (5). A lipopolysaccharide such as endotoxin is nearly exclusively cleared by Kupffer cells, whereas a mucopolysaccharide such as heparin is selectively internalized by endothelial cells (18). The lysosomal enzymes necessary for the degradation of these macromolecules include the proteolytic enzyme cathepsin D and the glycosidases β-glucuronidase and β-acetylglucosaminidase. The high specific activities of these enzymes in Kupffer and endothelial cells indicate a great capacity of these cells for the degradation of the macromolecules mentioned. It is of interest to note that arylsulphatase B which is involved in the degradation of mucopolysaccharides is indeed highly enriched in endothelial cells.

With respect to the high level of lysosomal glycosidases in sinusoidal cells, it is possible that they are partially of extracellular origin. Sinusoidal cells are capable of reducing the levels of the glycoproteins β-glucuronidase and β-acetylglucosaminidase in the serum (4,5,19). Since the endocytosed lysosomal enzymes do not undergo rapid catabolism (19,20), it seems reasonable to assume that the activities of β-glucuronidase and β-acetylglucosaminidase in Kupffer and endothelial cells may partially result from enzyme molecules acquired from the plasma. However, the extent of the possible contribution of these extracellular molecules to the endogenous enzyme activity is completely unknown at present.

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