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Activity of the lysosomal cysteine proteinases (cathepsin B,H,L) and a metalloproteinase (MMP-7-ase) in the serum of cystic fibrosis homozygous children

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Lysosomal cysteine proteinase (cathepsin B, H, L) and metalloproteinase (MMP-7-ase) activities were measured from serum of 19 cystic fibrosis (CF) homozygotes and of 13 healthy children, as control group.

The activity of cathepsin B and H significantly increased in the CF-group.

Introduction

The plasma lysosomal hydrolases, arginine esterase and IRT (immuno reactive trypsin) had been investigated in patients suffering from cystic fibrosis (CF); the latter was recommended for the screening of CF [1–6].

The diminished activity of the pancreatic enzymes is known as a leading laboratory finding in CF. Koheil et al [7] have published diminished carboxipeptidase-B-like activity among the intracellular proteases.

The cysteine proteinases (CB, CH, CL) and the metalloproteinase (MMP-7-ase) belong to the intracellular proteinases. These cysteine proteinases are lysosomal enzymes (Table I) [8–9], while MMP-7-ase is a cytosol one [10].

Earlier the elevated activity of the cysteine proteinases was investigated in the muscle tissue of dystrophic animals or of human myopathic patients [10–12].

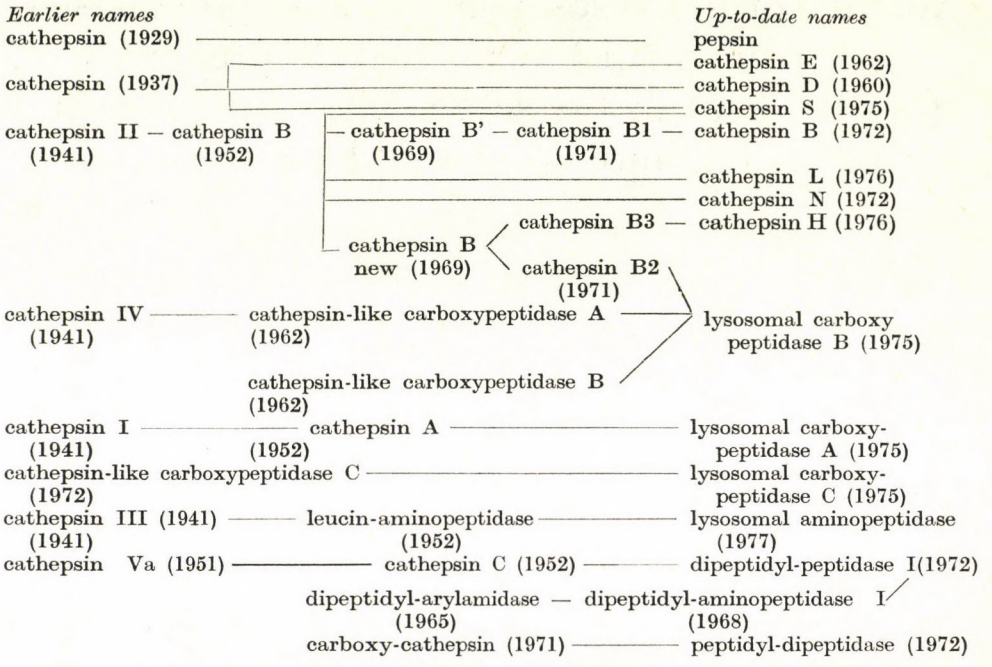
There are no data available about the cysteine proteinases or metalloproteinase of CF patients, that was the reason of our investigations.

Material and Methods

The activity of cathepsin B, H and L (CB, CH, CL) as lysosomal cysteine proteinase, MMP-7-ase as a metalloproteinase was measured in the serum of 19 CF homozygotes and in 13 healthy control children (4 months — 15 y.). Cysteine proteinase activities were measured by the method of Kirschke et al [12] and MMP-7-ase activity was determined by the method of Nakajima et al [13] for measurement of cathepsin G activity by Sohár et al [10] modified to MMP-7-ase activity using Suc-Ala-Ala-Pro-Phe-AMC (AMC means 7-methyl-4-amino coumarynil) as substrate. The conditions of assays are summarized

TABLE I

Nomenclature of the intracellular proteases (according to Barrett, modified)



BARRETT AJ. (ed): *Proteinases in Mammalian Cells and Tissues.* North-Holland Publ. Co., Amsterdam (1977)

TABLE II

Biochemical conditions of assays of examined enzymes

Name	EC. No.	Substrate	Concentration in assay, μM	pH	Buffer	Inhibitor	Catalytic group
Cathepsin B	3.4.22.1	Z-Arg-Arg--MCA	5.0	6.0	phosphate	chloroqine	-SH 2 mM
Cathepsin H	3.4.22.16	Arg-MCA	20.0	6.8	phosphate	Leu-CH_2-Cl	-SH 2 mM
Cathepsin L	3.4.22.15	Z-Phe-Arg--MCA	5.0	5.5	acetyte	Z-Phe-Phe--CHN_2	-SH 2 mM
MMP-7-ase	3.4.24	Suc-Ala-Ala-Pro-Phe-MCA	5.0	7.5	Tris-HCl	EDTA	metallo ion

MCA: 7-methyl-coumarynil-4-amide, Arg: arginine, Phe: phenylalanine, Ala: alanine, Pro: proline
S: bensyloxycarbonyl, Leu: leucine,
Suc: succinyl

TABLE III

The values of the activities of the serum cathepsin B, H, L and of the metalloproteinase (MMP-7-ase) in the group of CF homozygotes and of the control group

	Cathepsin = C mU/ml			MMP-7-ase
	CB	CH	CL	
CF homozygotes				
n = 19				
X =	17.39	123.65	1.66	7.67
S.D. ±	6.51	25.75	1.66	2.39
	$p < 0.01$	$p < 0.01$	$p = 0.36$	$p = 0.67$
	$T = 3.32$	$T = 3.64$	$U^* = 64.0$	$T = -0.82$
Control group				
n = 13				
X =	10.26	89.6	1.3	8.4
S.D. ±	3.7	33.6	0.83	2.61

U = μmol AMC/min
U* = -Wilcoxon test

in Table II. The liberated AMC was measured fluorimetrically (Ex.: 360 nm, Em.: 460 nm) with a Hitachi 650–10 (Tokyo, Japan) spectrofluorimeter. Substrates were supplied from Enzyme System (Livermore, CA).

The correlation between the clinical stages (in Schwachman score) and the investigated enzyme activities, and between activities of the cysteine proteinases and of the MMP-7-ase was determined by linear correlation coefficients.

RESULTS

The values of serum cysteine- and metallo-proteinase activities of CF homozygotes and of control group are summarized in Table III. The activity of CB and CH significantly increased in the CF homozygotes' group, while the activity of CL and MMP-7-ase did not change. The serum cysteine proteinases, the MMP-7-ase and the clinical stages did not correlate significantly according to the determination of the linear correlation coefficients.

DISCUSSION

The cathepsin B, H, L as cysteine proteinases can be activated with SH-group containing reagents or can be inhibited by SH-blockers.

Among the cysteine proteinases the CB and CH have showed significantly elevated activity in the serum of CF patients. The latter enzymes are localized into the lysosomes.

The metalloproteinase MMP-7-ase as a cytosol enzyme and the CL belonging to the cysteine proteinases did not show any enhancement of activity. The latter fact might be explained by the effect of the serum specific protease inhibitors.

The presence of cysteine proteinase inhibitors in the human muscle tissue

[14] and of endogenic cysteine proteinase inhibitors in the spleen has been proved [15]. There are no data about the cysteine proteinase inhibitors of CF patients which prompted us to determine the capacity of the latter ones in future.

Borgström et al [2] detected increased level of the serum immune reactive trypsin (IRT), chymotrypsin activity and of pancreatic trypsin inhibitor from the umbilical venous blood of CF homozygous newborns.

Our results show that cathepsin B and H may play a role in the faster protein degradation in patients having CF.

We are going to investigate the activity of the serum cysteine proteinases in the cases of the CF obligatory heterozygotes (parents), too.

We can conclude that the values of the cathepsin B and H activities in serum may be useful as complementary biochemical data of the laboratory diagnosis of CF.

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