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Relation of lysosomalexoglycosidases activity in serum of patients fed parenterally with nutrient mixtures – preliminary study

Mieszaniny żywieniowe a aktywność egzoglikozydaz lizosomalnych w surowicy krwi chorych żywionych parenteralnie – doniesienie wstępne

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Key words

N-acetyl-β-D-hexosaminidase, β-galactosidase, α-mannosidase, α-fucosidase, β-glucuronidase, parenteral nutrition, serum

Słowa kluczowe

N-acetylo-β-D-heksozoaminidaza β-galaktozydaza, α-mannozydaza, α-fukozydaza, β-glukuronidaza, żywienie parenteralne, surowica krwi

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S u m m a r y

Introduction. Many clinical situations as: complications of intestinal obstruction, especially after operations on the gastrontestinal tract, extensive burn, multiorgans injury, create the necessity for application of parenteral nutrition. In parenteral nutrition, improperly chosen nutrients may cause numerous metabolic complications including disorders of liver, lung and kidney function.

Aim. Therefore, we decided to determine the influence of the composition of parenterally administered four different types of nutrient mixtures on the profile of serum activity of lysosomalexoglycosidases: N-acetyl-β-D-hexosaminidase (HEX), β-galactosidase (GAL), α-mannosidase (MAN), α-fucosidase (FUC) and β-glucuronidase (GLU).

Material and methods. Blood samples were collected from 23 patients before, and 5 and 10 days after parenteral nutrition with four different diets. Exoglycosidases activity in serum was determined by the colorimetric method of Zwierzet al.

Results. Conducted examination proved no significant differences between concentration of lysosomalexoglycosidases activity in serum of patients fed with different composition of nutrient mixtures.

Conclusions. Parenteral nutrition (up to 10 days) did not influences serum activity of lysosomalexoglycosidases.

S t r e s z c z e n i e

Wstęp. Wiele sytuacji klinicznych jak:powikłania w postaci niedrożności przewodu pokarmowego, szczególnie po operacjach na przewodzie pokarmowym, rozległe oparzenia, urazy wielonarządowe stwarzają konieczność zastosowania żywienia pozajelitowego. W żywieniu parenteralnym niewłaściwie dobrane składniki odżywcze mogą powodować szereg powikłań metabolicznych, w tym zaburzenia funkcji wątroby, płuc i nerek.

Cel pracy. Postanowiliśmy określić wpływ składu przetaczanych czterech mieszanin żywieniowych na profil surowiczych aktywności egzoglikozydaz lizosomalnych: N-acetylo-β-D-heksozoaminidazy (HEX), β-galaktozydazy (GAL), α-mannozydazy(MAN), α-fukozydazy (FUC) and β-glukuronidazy (GLU).

Materiał i metody. Krew pobrano od 23 pacjentów przed rozpoczęciem żywienia parenteralnego, w 5 oraz 10 dobie alimentacji dożylnej czterema różnymi mieszaninami żywieniowymi. Aktywność egzoglikozydaz lizosomalnych w surowicy krwi oznaczano metodą kolorymetryczną Zwierza i wsp.

Wyniki. Przeprowadzone badanie wskazuje na brak znamiennych statystycznie różnic pomiędzy stężeniem aktywności egzoglikozydaz lizosomalnych w surowicy krwi pacjentów żywionych przy użyciu czterech mieszanin żywieniowych o różnym składzie.

Wnioski. Krótkotrwale żywienie pozajelitowe (do 10 dni)nie wpływa istotnie na aktywność egzoglikozydaz lizosomalnych surowicy krwi.

INTRODUCTION

Parenteral nutrition is connected with total exclusion of regulatory function of gastrointestinal tract from systemic homeostasis support (1). Liver dysfunction is the most frequently described complication connected with parenteral nutrition (2). Disorders of liver function regard 15-40% (3, 4) and even 30-90% of patients fed parenterally for more than 2 weeks (5). During parenteral nutrition, nutrient mixture is administered intravenously and patient has to absorb or excrete it. Improperly chosen nutrients may lead to large amount of metabolic complications (6). Many metabolic complications may be connected with disorders of glycoconjugates (glycoproteins of cell membranes and biological fluids, glycolipids of cell membranes and proteoglycans of cell membranes and intracellular substance) metabolism (7-9). Considering the influence of parenteral nutrition on the activity of lysosomal exoglycosidases: N-acetyl-β-D-hexosaminidase (HEX), β-galactosidase (GAL), α-mannosidase (MAN), α-fucosidase (FUC) and β-glucuronidase (GLU) (10-12), we decided to estimate the influence of composition of administered nutrient mixture on the profile of lysosomal exoglycosidases.

AIM

Aim of our paper was determination of the influence of the composition of parenterally administered four different types of nutrient mixtures on the profile of serum activity of lysosomalexoglycosidases: N-acetyl-β-D-hexosaminidase (HEX), β-galactosidase (GAL), α-mannosidase (MAN), α-fucosidase (FUC) and β-glucuronidase (GLU).

MATERIAL AND METHODS

The study has received approval from the Bioethical Commission of the Medical University of Bialystok (No. R-I-003/320/2006).

The group of examined patients has been fed parenterally in the 1st Department of General and Endocrinological Surgery, Medical University of Białystok. The study included 23 patients (8 women and 15 men) aged 22-82, average age 57.1 ± 19.37. The criteria excluding patients from study group were: diabetes, obesity, alcoholic disease, renal and liver dysfunctions and septic complications connected with introduction of the catheter to the central veins. Parenteral nutrition was intravenously administered in an all-in one 24 hour procedure using an infusion pump for maintaining a constant rate of nutrient delivery. The nutrient mixture was made up at the hospital's dispensary and patients received 4 types of nutritional formulae as follows:

A. 15% Aminoplasmal E (1000 ml), 10% Intralipid (500 ml), 20% Glucose (1000 ml), Gensulin R (36j), 15% KCl (40 ml), 20% $MgSO_4$ (10 ml), Addamel (1 vial), Addiphos (1 vial) and Cernevit (1 vial);
B. 10% Aminoplasmal hepa (1000 ml), 10% Intralipid (500 ml), 10% Glucose (500 ml), Gensulin R (36j), 15% KCl (40 ml), 20% $MgSO_4$ (10 ml), Addamel (1 vial), Addiphos (1 vial) and Cernevit (1 vial);
C. 15% Aminoplasmal E (500 ml), 20% Clinoleic (100 ml), 20% Glucose (1000 ml), Gensulin R (36j), 15% KCl (40 ml), 20% $MgSO_4$ (10 ml), Addamel (1 vial), Addiphos (1 vial) and Cernevit (1 vial);
D. 10% Aminosteril KE (500 ml), 20% Clinoleic (100 ml), 20% Glucose (1000 ml), Gensulin R (40j), Vit. B1 (100 mg), 20% $MgSO_4$ (10 ml), Addamel (1 vial), Addiphos (1 vial) and Cernevit (1 vial).

Nutrient mixture "A" was administered to 6 patients, "B" to 5 patients, "C" to 7 and mixture "D" to 5 patients.

Serum samples were collected at 3 time points; before the beginning of parenteral nutrition (baseline), 5 and 10 days thereafter. The samples were then centrifuged for 20 minutes at 4000 x g, 4°C and supernatants were suitably aliquoted and stored in Eppendorf tubes at -80°C ready for use. Comparing HEX, GAL, MAN, FUC and GLU activities after 5 and 10 days of parenteral nutrition with the baseline thus allowed any effects of the patient's illness *per se* on these enzyme activities, to be excluded.

The time of sampling was in the first instance based on recommendations for monitoring parenteral nutrition at its early stages (i.e. after 3-5 days) and comparing these with baseline results, thereby allowing organ function to be assessed as well as any modifications to the nutrient formulations to be made whenever required (13). In addition, studies have shown that after 9-10 days of parenteral nutrition in rats, the activity of lysosomal exoglycosidases changes when measured in liver, kidney and spleen homogenates (14-16).

Activities of HEX, GAL, MAN, FUC and GLU were measured, in duplicates, by the method of Zwierz et al. (17) adapted to the microtiter plate (NUNC) format. The released p-nitrophenol was determined by its absorbance at 405 nm and the actual amounts released p-nitrophenol were calculated from a calibration curve using a microplate reader (Elx 800 TM, Bio-Tek Instruments, Inc. Vermont, USA).

STATISTICAL ANALYSIS

Changes of the exoglycosidase's activity across time were analysed by Friedman analysis of variance (ANOVA) and Kendall concordance. For comparisons between groups (A, B, C, D), analysis of variance (ANOVA) with NIR post-hoc tests has been applied.

RESULTS

Serum activity analysis of HEX, GAL, MAN, FUC and GLU in patients fed parenterally revealed only slight tendency to decrease after 5 days and further slight tendency to increase after 10 days (tab. 1). Conducted study proves lack of significant differences ($p = 0.055197 - 1.000000$) between concentration of serum activity of lysosomal exoglycosidases in patients fed parenterally with nutrient mixtures of different composition (tab. 2-6).

DISCUSSION

It was reported that nutrients may regulate the activity of lysosomal exoglycosidases e.g. some groups of plants (Rosaceae, Leguminosae), containig D-saccharic acid – the progenitor of D-saccharic-1,4-lactone, inhibit β-glucuronidase (18). In the literature, we had not found any records regarding influence of nutrient mixtures on the activity of examined lysosomal exoglycosidases.

In this study, mixtures applied during parenteral nutrition contained lipid emulsions: Intralipid and Clinoleic which consist of long-chain fatty acids. Clinoleic, unlike Intralipid, which is based strictly on soya, consists also of olive oil and has lower level of polyunsaturated fatty acids. Beneficial influence of Clinoleic is connected with its antioxidant activity, protection of lipid membranes exposed to damage and limitation of the number of progenitors of the synthesis of immunosuppressive and proinflammatory compounds (19). Thus, it may be assumed that patients fed parenterally with Clinoleic would have lower concentration of lysosomal exoglycosidases in serum. Research of Roth et al., conducted on rats, proved that emulsion containing long-chain fatty acids reveals higher tendency to deposit in Kupffer's cells than mixtures containing long- and medium-chain fatty acids (15). There is no research estimating whether emulsions of long-chain fatty acids and long-chain fatty acids with decreased amount of polyunsaturated acids reveal similar tendency to deposit in hepatocytes and whether they may cause differences in exoglycosidases activity. It may be assumed that glucose added to nutrient mixtures at different concentrations (in our study group 10 and 20%) may also cause changes in serum activity of lysosomal exoglycosidases. It has been proved that excessive supply of glucose may intensify processes of liponeogenesis. Lipogenesis, connected with high production of CO2 may lead to respiratory failure (20) and therefore to hypoxia and the increase of permeability of lysosomal membranes for enzymes (21). However, the situation when the liver is not able to release created triacylglycerols to the blood may lead to steatosis (20, 22) which may be reflected in the increase of lysosomal exoglycosidases activity observed in steatosis (23).

Characteristic changes in lysosomes function were observed after administration of the low protein diet to examined animals. Protein deficit in diet was expressed by activation of the majority of lysosomal hydrolases, mainly peptidases, in liver, kidneys and spleen of rats (24). Results obtained by Poriadkova et al. also seem to be interesting. They proved that the level of hydrolysis of protein preparations administered to animals intravenously, does not influence the activity of lysosomal hydrolases in liver, kidneys and spleen (25).

In our group of examined patients it is impossible to undoubtedly estimate the influence of particular components of nutrient mixture on lysosomal exoglycosidases activity. In order to determine the influence of particular components of parenteral mixture on serum lysosomal enzymes, the group of patients should be gathered where nutrient mixture with periodic change of one component would be applied which is very difficult, according to ethical issues. Results of our research indicate the lack of significant differences between concentration of serum activity of lysosomal exoglycosidases in patients fed parenterally with nutrient mixtures of different composition (tab. 2-6). Thus, it may be assumed that the process of parenteral nutrition with the omission of gastrointestinal tract, and not the composition of nutrient mixtures, may cause of changes of lysosomal exoglycosidases activity.

Parenteral nutrition is a method of clinical nutrition which interferes patient's metabolism. During the application of nutrient mixtures of different composition, the tendency to decrease of serum lysosomal exoglysidases czy nie exoglycosidases? activity after 5 days, as well as tendency to increase after 10 days have been stated (tab. 1). Results of our previous research suggest significant influence of parenteral nutrition on the activity of serum lysosomal exoglycosidases (10-12). Both, current and previous research indicate that intravenous alimentation may influence enzymatic system of lysosomes, however there is no final proof for above statement.

CONCLUSIONS

Short period of parenteral nutrition (up to 10 days) did not significantly changes serum activity of lysosomal exoglycosidases.

Table 1. Activity of HEX. GAL. MAN. FUC and GLU in serum of patients fed intravenously with nutrient mixtures of different composition.

SERUM													
Enzyme subsequent day of parenteral nutrition		Nutrient mixture A			Nutrient mixture B			Nutrient mixture C			Nutrient mixture D		
		average activity (nmol/ml/min)	median	standard deviation SD	average activity (nmol/ml/min)	median	standard deviation SD	average activity (nmol/ml/min)	median	standard deviation SD	medium activity (nmol/ml/min)	median	standard deviation SD
HEX	**0**	41.57	37.36	13.48	29.40	29.11	2.05	35.61	36.82	11.00	27.59	24.26	12.65
	5	36.59	31.61	14.83	24.35	21.86	5.50	24.91	25.03	2.87	18.73	16.78	7.32
	10	50.98	50.98	16.25	32.18	31.55	4.33	31.87	35.68	9.52	20.61	19.31	5.01
GAL	**0**	10.53	11.88	2.16	8.10	8.98	2.81	8.22	9.90	3.21	5.88	4.62	2.76
	5	5.50	5.62	1.66	6.02	5.38	1.23	5.96	5.70	1.66	6.08	5.79	2.18
	10	6.41	6.41	1.16	9.05	8.12	2.96	5.69	5.15	1.62	8.46	6.58	5.36
MAN	**0**	12.77	13.38	3.78	9.10	7.70	2.63	9.03	10.42	3.10	6.37	5.60	2.38
	5	7.15	7.89	2.04	6.38	5.57	1.43	7.61	7.33	3.05	6.64	6.11	2.23
	10	6.60	6.60	0.48	8.96	9.30	2.90	7.72	7.85	1.71	6.09	6.10	0.90
FUC	**0**	11.92	11.77	0.45	13.53	13.10	2.77	12.98	11.81	4.33	12.26	10.73	3.68
	5	10.08	8.06	3.64	11.90	13.22	4.99	10.37	9.65	3.77	10.25	9.50	4.37
	10	7.33	7.33	2.78	10.91	12.07	3.84	9.40	8.18	4.42	7.89	7.61	3.78
GlU	**0**	15.49	15.80	4.23	14.26	14.36	2.65	14.73	14.77	4.82	14.17	13.39	2.00
	5	12.72	13.29	2.68	11.72	10.76	2.45	12.58	14.36	4.88	10.75	10.77	1.39
	10	13.73	13.73	0.68	14.40	14.21	3.17	14.42	14.71	2.70	13.41	11.81	4.05

Table 2. The comparison of serum activity of HEX in patients fed parenterally with nutrient mixtures of different composition.

Parenteral nutrition		**Before**				**5 days**				**10 days**			
	Mixtures	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Before	**A**												
	B	1.000000											
	C	1.000000	1.000000										
	D	1.000000	1.000000	1.000000									
	A	1.000000	1.000000	1.000000	1.000000								
5 days	**B**	0.714679	1.000000	1.000000	1.000000	1.000000							
	C	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000						
	D	0.060032	1.000000	0.341536	1.000000	0.586122	1.000000	1.000000					
	A	1.000000	1.000000	1.000000	0.384613	1.000000	0.197647	0.341886	0.071548				
10 days	**B**	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.634404	1.000000			
	C	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		.
	D	0.125308	1.000000	0.773621	1.000000	1.000000	1.000000	1.000000	1.000000	0.055197	1.000000	1.000000	

Table 3. The comparison of serum activity of GAL in patients fed parenterally with nutrient mixtures of different composition.

Parenteral nutrition		Before				5 days				10 days			
	Mixtures	A	B	C	D	A	B	C	D	A	B	C	D
Before	A												
	B	1.000000											
	C	1.000000	1.000000										
	D	0.256452	1.000000	1.000000									
5 days	A	0.211467	1.000000	1.000000	1.000000								
	B	0.551455	1.000000	1.000000	1.000000	1.000000							
	C	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000						
	D	0.682960	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000					
10 days	A	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000				
	B	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000			
	C	0.505794	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		
	D	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Table 4. The comparison of serum activity of MAN in patients fed parenterally with nutrient mixtures of different composition.

Parenteral nutrition		Before				5 days				10 days			
	Mixtures	A	B	C	D	A	B	C	D	A	B	C	D
Before	A												
	B	1.000000											
	C	1.000000	1.000000										
	D	0.110389	1.000000	1.000000									
5 days	A	1.000000	1.000000	1.000000	1.000000								
	B	0.102885	1.000000	1.000000	1.000000	1.000000							
	C	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000						
	D	0.191785	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000					
10 days	A	0.727187	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000				
	B	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000			
	C	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		
	D	0.108251	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Table 5. The comparison of serum activity of FUC in patients fed parenterally with nutrient mixtures of different composition.

Parenteral nutrition		Before				5 days				10 days			
	Mixtures	A	B	C	D	A	B	C	D	A	B	C	D
Before	A												
	B	1.000000											
	C	1.000000	1.000000										
	D	1.000000	1.000000	1.000000									
5 days	A	1.000000	1.000000	1.000000	1.000000								
	B	1.000000	1.000000	1.000000	1.000000	1.000000							
	C	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000						
	D	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000					
10 days	A	1.000000	0.464280	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000				
	B	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000			
	C	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		
	D	1.000000	0.614645	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Table 6. The comparison of serum activity of GLU in patients fed parenterally with nutrient mixtures of different composition.

Parenteral nutrition		Before				5 days				10 days			
	Mixtures	A	B	C	D	A	B	C	D	A	B	C	D
Before	A												
	B	1.000000											
	C	1.000000	1.000000										
	D	1.000000	1.000000	1.000000									
5 days	A	1.000000	1.000000	1.000000	1.000000								
	B	1.000000	1.000000	1.000000	1.000000	1.000000							
	C	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000						
	D	0.560805	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000					
10 days	A	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000				
	B	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000			
	C	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		
	D	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Aknowledgements

The authors wish to express their thanks to Professor Jacek Dadan, MD, the Head of the Department of General and Endocrinological Surgery, Medical University of Białystok, for providing the study material.

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