EDETATE CALCIUM DISODIUM CHELATING AGENT INHIBITS *IN VITRO* SERUM LIPASE AND AMYLASE ACTIVITY IN ACUTE PANCREATITIS

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Abstract: *The good results obtained with chelating agent in the treatment of acute pancreatitis associated with saturnin colic suggested the question if they are not due to the enzymatic inhibition made by Edetate, Calcium Disodium. We have studied in vitro the effect of Edetate Calcium Disodium, on the activity of the pancreatic lipase and amylase. We used a group of 41 patients. The cases included acute pancreatitis of biliary (the most frequent one), alcoholic, metabolic, postoperative, and idiopathic etiology. Edetate Calcium Disodium was used in the dilution of 0,014 mg/mL serum. The research pointed out the statistically significant reduction of the enzyme level.*

Cuvinte cheie: *calciumdinatriumedetat, efect in vitro, pancreatită acută*

Rezumat: *Rezultatele bune obținute cu agent chelator în tratamentul pancreatitei acute asociate colicii saturnine au ridicat problema dacă ele nu sunt cumva datorate inhibiției enzimatice produse de calciumdinatriumedetat. Noi am studiat in vitro efectul calciumdinatriumedetat-ului asupra activității lipazei și amilazei pancreatice. Am folosit un lot de 41 de pacienți. Cazurile au inclus pancreatite acute de etiologie biliară (cele mai frecvente), alcoolică, metabolică, postoperatorie și idiopatică. Calciumdinatriumedetat-ul a fost utilizat în diluție de 0,014 mg/mL de ser. Studiul a arătat scăderea statistic semnificativă a nivelelor enzimatice.*

INTRODUCTION

The treatment of acute pancreatitis is still a matter of controversy as few of the therapeutic means that have been tested so far proved to be efficient. We have previously studied 31 cases of acute pancreatitis associated with saturnine colics (an acute digestive manifestation of lead poisoning). We have ascertained a favourable evolution of the patients' state when the treatment consisted of a chelating agent (Edetate Calcium Disodium,), which accompanied the classical therapy. These favourable results led us to the assumption that the therapy with chelating agents might not only eliminate the lead (an etiological factor of pancreatitis), but also inhibit the pancreatic enzymes. Some of them contain metals in their structure. Calcium and magnesium are activating agents of some pancreatic enzymes. The present article studies the *in vitro* effect of the therapy with Edetate Calcium Disodium on the pancreatic lipase and amylase in acute pancreatitis with other etiology than the saturnine one.

PURPOSE

The present article studies the in vitro effect of the therapy with Edetate Calcium Disodium on the activity of the pancreatic lipase and amylase in acute pancreatitis with other etiology than saturnine intoxication.

METHODS

A group of 41 consecutive patients with acute pancreatitis has been studied during their hospitalisation in the Emergency Departmental Clinical Hospital of Sibiu. The diagnosis of acute pancreatitis has been established on clinical, imagistic (ultrasonograhpy, computer tomography) and biological data. The pancreatic lipase has been measured by the Neumann and Ziegehorn method, with normal values of up to 190 U/L. The pancreatic amylase has been determined by the Smith and Roe method, with the normal values in the blood varying between 60-200 amylasic U/L. The pancreatic enzymes were studied after blood had been gathered, at the beginning of hospitalization, before any drug had been given to them. The enzymes were again measured in a second serum sample one hour after the serum had been treated with Edetate Calcium Disodium (the substance was diluted by 0,014 mg/mL serum). Serum enzymes were also determined in the first serum sample which had been incubated for one hour. The statistical significance of the results was assessed by the Student's t test. The serum lipase was determined in 31 patients and serum amylase in 16 patients.

RESULTS

The distribution of cases according to age, etiology, gender and results of the experiments is presented in tables no. 1 and 2 and 4. The average age of the examined group was 54,8 years. A number of 21 (51,2%) patients were women, and 20 (48,8%) were men. From the point of view of etiology, biliary acute pancreatitis was the most frequent one (20 patients), followed by the alcoholic one (16 cases) and by a small number of idiopathic (2 cases), metabolic (2 cases) and postsurgical pancreatitis (1 case).

The serum pancreatic enzymes values did not modify at one hour of incubation after the first sampling (without Edetate Calcium Disodium administration). After chelating agent administration, it was observed a statistically significant decrease of pancreatic enzymes comparing with their values from the first sampling and with their values in serum collected

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at one hour from the first sampling. Only in one patient (3.22%), lipase value was not normalized, and in two patients (12.5%) serum amylase concentrations were not normalized, although they significantly decreased.

Table no. 1. Repartition of the patients with acute pancreatitis regarding age, gender, etiology and the values of the serum lipases (U/L) at the 4 determinations during the study

Current number	Age	Gender	Etiology	At admission	Sample taken at one hour after admission	After incubation Sample taken at one hour after the first sample	After treatment with CaNa2EDTA
1	49	F	Alcoholic	828	831	826	46,6
2	60	F	Biliary	868	861	867	57,6
3	58	F	Biliary	1050	1059	1048	170,0
4	61	F	Biliary	4615	4450	4370	156,8
5	50	F	Biliary	4270	4281	4269	47,5
6	55	M	Postoperative	2715	2724	2715	72,0
7	41	M	Alcoholic	680	671	679	42,0
8	51	M	Alcoholic	5704	5290	4950	164,0
9	39	F	Idiopathic	264	270	264	42,0
10	54	F	Biliary	1705	1711	1704	76,0
11	41	F	Biliary	604	618	604	28,0
12	43	F	Biliary	268	260	267	47,6
13	48	F	Biliary	397	381	396	14,0
14	65	M	Biliary	644	633	643	18,0
15	60	F	Biliary	6580	6130	5730	184,0
16	57	M	Postoperative	800	791	799	27,0
17	48	M	Alcoholic	1190	1181	1188	84,0
18	56	F	Biliary	1724	1728	1722	62,0
19	62	M	Biliary	627	635	627	21,5
20	53	M	Alcoholic	290	298	189	84,0
21	55	M	Alcoholic	1520	1514	1519	72,0
22	58	F	Biliary	417	409	416	17,6
23	49	M	Hiperlipemia	500	509	498	40,0
24	43	M	Alcoholic	1120	1136	1119	48,0
25	39	M	Idiopathic	4480	4230	3870	262,2
26	61	M	Alcoholic	1520	1517	1519	19,8
27	51	F	Biliary	4438	4130	4020	56,0
28	68	M	Alcoholic	300	310	299	19,6
29	38	F	Biliary	476	479	474	56,0
30	60	M	Alcoholic	1050	1041	1048	84,0
31	48	F	Alcoholic	1500	1507	1499	19,8
Average				**1247,6**	**1215,2**	**1176,2**	**53,9**

Table no. 2. Repartition of the patients with acute pancreatitis regarding age, gender, etiology and the values of the serum amylases (U/L) at the 4 determinations during the study

Current number	Age	Gender	Etiology	t admissio	Sample taken at one hour after admission	After incubation Sample taken at one hour after the first sample	After treatment with CaNa2EDTA
1	49	F	Alcoholic	454	480	453	80
2	60	F	Biliary	500	509	498	200
3	58	F	Biliary	380	369	379	60
4	61	F	Biliary	4000	3742	3792	420
5	50	F	Biliary	2060	2041	2059	200
6	55	M	Postopera	2350	2303	2349	80
7	55	F	Biliary	710	715	709	132
8	42	M	Alcoholic	330	339	328	30
9	58	F	Biliary	350	299	349	60
10	49	M	Alcoholic	370	361	370	60
11	52	M	Alcoholic	484	480	483	80
12	41	F	Biliary	500	509	499	200
13	39	F	Biliary	700	715	697	70
14	52	M	Alcoholic	3500	3323	3263	400
15	46	F	Biliary	500	499	499	31
16	61	M	Alcoholic	480	488	478	60
Average				**1104,3**	**1073,3**	**1075,3**	**135,2**

Notations: Ho = null hypothesis; N = the lot volume (number of patients); L = number of liberty grades (L = N1+N2 -2); p = signification grade (of the risk) ; 1-p = 0,99 confidence interval; (t1,t2) = the interval of the acceptance of the Ho hypothesis; Tc = the calculated value of the t Student test corresponding to the type of Ho announced null hypothesis; Tc = (m1-m2)/sqrt((N1-1)*V1+(N1-1)*V2)*sqrt(L/(1/N1+1/N2)); m1 = the average values of the samples taken at admission; m2 = the average values of the samples taken at one hour after admission; m3 = the average values of the samples after incubation, taken at one hour after the first sample; m4 = the average values of the samples taken after treatment with CaNa2EDTA; V1 = standard dispersion (square standard deviation) in the first batch which is compared; V2 = standard dispersion in the second batch which is compared.

Table no. 3. Serum lipases: The t Student test for comparing two averages from two populations normally distributed, having unknown, equal, standard deviations

Parameters	Comparing m1 with m2	Comparing m1 with m3	Comparing m1 with m4	Comparing m3 with m4
Ho	m1=m2	m1=m3	m1=m4	m3=m4
N	31	31	31	31
L	60	60	60	60
p	0,01	0,01	0,01	0,01
(t1,t2)	(- 2,66 ;2,66)	(- 2,66 ;2,66)	(- 2,66 ;2,66)	(- 2,66 ;2,66)
Tc	0,155	0,228	5,176	5,469
Conclusion	0,155 c (-2,66 ;2,66) Admitted Ho	0,228 c (-2,66 ;2,66) Admitted Ho	5,176 c (-2,66 ;2,66) Reject Ho	5,469 c (-2,66 ;2,66) Reject Ho

Table no. 4. Serum amylases: The t Student test for comparing two averages from two populations normally distributed, having unknown, equal, standard deviations

Parameters	Comparing m1 with m2	Comparing m1 with m3	Comparing m1 with m4	Comparing m3 with m4
Ho	m1=m2	m1=m3	m1=m4	m3=m4
N	16	16	16	16
L	30	30	30	30
p	0,01	0,01	0,01	0,01
(t1,t2)	(- 2,75 ;2,75)	(- 2,75 ;2,75)	(- 2,75 ;2,75)	(- 2,75 ;2,75)
Tc	0,075	0,07	3,226	3,305
Conclusion	0,075 c (-2,75 ;2,75) Admitted Ho	0,07 c (-2,75 ;2,75) Admitted Ho	3,226 c (-2,75 ;2,75) Reject Ho	3,305 c (-2,75 ;2,75) Reject Ho

Notations: Ho = null hypothesis; N = the lot volume (number of patients); L = number of liberty grades (L = N1+N2 -2); p = signification grade (of the risk) ; 1-p = 0,99 confidence interval; (t1,t2) = the interval of the acceptance of the Ho hypothesis; Tc = the calculated value of the t Student test corresponding to the type of Ho announced null hypothesis; Tc = (m1-m2)/sqrt((N1-1)*V1+(N1-1)*V2)*sqrt(L/(1/N1+1/N2)); m1 = the average values of the samples taken at admission; m2 = the average values of the samples taken at one hour after admission; m3 =the average values of the samples after incubation, taken at one hour after the first sample; m4 =the average values of the samples taken after treatment with CaNa2EDTA; V1 = standard dispersion (square standard deviation) in the first batch which is compared; V2 = standard dispersion in the second batch which is compared.

DISCUSSIONS AND CONCLUSIONS

Few medical methods of treatment have proved their efficiency in the treatment of acute pancreatitis. Therefore, new methods of treatment are tested at present. The good results that have been obtained in the treatment of acute pancreatitis associated with saturnine colic with chelating agents suggest that they could also depend on the enzyme inhibition caused by Edetate Calcium Disodium. The current study, made *in vitro* on 41 patients, brought arguments to confirm this hypothesis. From a statistical point of view, we noted a significant decrease of the pancreatic enzyme activity after the treatment with Edetate Calcium Disodium.

It is well-known that some pancreatic enzymes contain metals in their structure (amylase contains zinc), others have calcium or zinc as a stabilizer. Lipase contains in its active centre a catalytic triad: asparaginic acid, histidine and serine. Asparaginic acid steal a proton from histidine, activating it. In turn, histidine catalytic active steals a proton from serine, increasing nucleofilia of serine rest. It acts on the carbonyl of an ester of substrate located in the active centre. It arises a tetraedric intermediary product of which will result an acyl-enzyme complex. By deacetilation, a hydrolysis step, it results a fatty acid and a free enzyme. The N-terminal domain of pancreatic lipase contains a Ca^{2+} binding site in a loop region.(1,2,3)

The pancreatic lipase is activated by calcium and magnesium.(4,5,6,7,8,9) In acute pancreatitis, the growth of amylase is a proof of enzymatic activation. On the other hand, lipase has an obvious role in the pathogenesis of the pancreatitic process. Lipase causes fatty necrosis, which may be local and / or metastatic. The enzyme alters adipocytes, the trigger events that cause cytosteatonecrosis.

Mechanisms by which Edetate Calcium Disodium inhibits pancreatic lipase require some clarifications. The substance forms chelates with di-and trivalent metals. Between minerals and metals that are chelated by this there are calcium and magnesium. It is likely that Edetate Calcium Disodium interferes lipase activation by calcium and magnesium.(10,11,12)

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