Intestinal Clearance of α_1-Antitrypsin
A Sensitive Method for the Detection of Protein-Losing Enteropathy

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Gastrointestinal loss of plasma is usually measured with radiolabeled macromolecules. These methods are expensive and cumbersome. The use of α_1-antitrypsin as an endogenous marker and the determinantion of α_1-antitrypsin fecal clearance enable the diagnosis of protein-losing enteropathy. α_1-Antitrypsin is measured in feces and blood by radial immunodiffusion, and the results are expressed as clearance. There is a significant correlation between α_1-antitrypsin fecal clearance and ^{51}Cr-plasma protein clearance ($r = 0.96$, $p < 0.001$). The sensibility of α_1-antitrypsin test compared to [^{51}Cr] is 93.3%, the specificity is 90%. The positive predictive value is 97.7%, the negative predictive value 75%. We found no α_1-antitrypsin in gastric juice of pH below 3. In vitro studies confirmed the destruction of α_1-antitrypsin in gastric juice of pH below 3. There is a slight decrease of α_1-antitrypsin concentration when stools are incubated at 37°C. In duodenal juice there is a small lessening of α_1-antitrypsin concentration after an incubation at 37°C for 1 h. In conclusion, the fecal clearance of α_1-antitrypsin seems to be an inexpensive and quite reliable test of protein-losing enteropathy.

Although measurement of fecal radioactive chromium after intravenous injection of radioactive chromium chloride is generally considered to be the most accurate test for detecting protein-losing enteropathy, the test is not widely used because of its poor acceptability by patients, physicians and clinical chemists.

For the patient the test involves inconveniently staying in the hospital for 6-10 days with daily collection of blood and feces, the use of radioactive products, and the expense of these procedures. For the physician the test prevents other diagnostic procedure, i.e., endoscopic investigation, barium enema, and other tests including some on blood samples. For the clinical chemist the test involves the use of a gamma counter and the manipulation of radioactive products.

Because of these disadvantages we have looked for an alternative method suggested by Crowsley and Elliot (4). α_1-antitrypsin (α_1-AT) was used as an endogenous marker instead of radiolabeled macromolecules. In a preliminary work (2) we have shown that the determination of α_1-antitrypsin intestinal clearance over a 10 day period could be a reliable index of protein-losing enteropathy. However, Haeney et al. (5) did not obtain correlation between fecal loss of α_1-antitrypsin and ^{51}Cr-albumin and have seriously questioned the validity of fecal α_1-antitrypsin determination.

The aim of the present study was to compare the results of intestinal clearance of plasma protein measured simultaneously with α_1-AT and radiochromium, and to test the resistance of α_1-AT in biologic fluids.

Materials and Methods

Patients

Fifty-five patients were simultaneously studied by both α_1-AT and ^{51}Cr-clearances. They were separated into three groups: inflammatory bowel diseases (n = 21), noninflammatory bowel diseases (n = 23), and miscellaneous (n = 6). The diagnoses were: (a) inflammatory bowel diseases: Crohn's disease (n = 15); colon alone (n = 6); small bowel + colon (n = 5); small intestine (n = 4); ulcerative colitis (n = 6). (b) Noninflammatory bowel disease: celiac disease (n = 2); anastomotic ulcer (n = 3); cirrhosis of the

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Table 1. Results of Protein Losses Study (ml/day)

Group I													
Inflammatory bowel diseases	[^{51}Cr]Cl	45	382	153	178	54	90	378	115	142			
	1 AT	23	200	73	120	14	25	200	60	40			
	[^{51}Cr]Cl	340	127	92	379	340	320	315	285	255	255	275	210
	1 AT	190	50	20	190	200	170	160	140	140	120	160	95
Group II													
Noninflammatory bowel diseases	[^{51}Cr]Cl	267	37	143	30	94	106	100	122	54	14,5	395	140
	1 AT	118	7	60	13	27	58	17	60	23	4	180	50
	[^{51}Cr]Cl	325	75	45	40	40	30	45	45	70	240	18	
	1 AT	160	15	12	17	15	8	17	20	20	100	7	
Group III													
Miscellaneous	[^{51}Cr]Cl	96	46	45	70	65	385						
	1 AT	15	10	7	13	18	212						
Group IV													
Normal subjects	[^{51}Cr]Cl	30	22	31	17	21							
	1 AT	8	6	12	3	7							

liver (n = 5); cirrhosis with hepatoma (n = 5); cirrhosis with tuberculosis of peritoneum and liver (n = 5); intestinal lymphangiectasia (n = 1); cardiac failure (n = 2). And (c) miscellaneous: small bowel lymphoma (n = 1); hypogammaglobulinemia (n = 1); radiation enteritis (n = 2); blind loop syndrome (n = 1); and Menetrier's disease (n = 1).

Methods

Clearance of ^{51}Cr-proteins and clearance of α_1-AT were simultaneously determined in 55 subjects. Each subject was studied for a period of 6 days with daily collection of feces and blood samples.

α_1-Antitrypsin determination. α_1-antitrypsin was measured by radial immunodiffusion. The immunodiffusion plates contained a monospecific antiserum against α_1-AT, M or LC partigen (kindly supplied by Behringwerke Laboratories). The precipitating ring was measured at the 72nd h. A reference curve was established with a standard solution for each batch of plates. The sera were kept at −20°C until assayed. They were diluted in NaCl (150 mM) at one-tenth. Five microliters of diluted sera were put into wells of M partigen plates. Feces samples were kept at −20°C until assayed and in varying proportions were diluted (usually one-third) in isotonic NaCl before being mixed for 60 min. After centrifugation at 1500 *g* for 20 min, 20 μl of the supernatant were put into wells of LC partigen plates. The following determinations were made daily for each subject: fecal volume (V) in mg/day; fecal α_1-AT concentration (F) in mg/100 ml, α_1-AT fecal loss/day (Q = F × V) in mg/day; α_1-AT serum concentration (P) in mg/100 ml; α_1-AT intestinal clearance (α_1-AT Cl) (Cl = V × F/P) in ml serum/day.

^{51}Cr clearance (13). Fifty microcuries of [^{51}Cr]Cl_3 (O.R.I.S. 91190 Gif-sur-Yvette, France) were injected intravenously 2 days before the beginning of the study. Radioactivity was measured in serum and feces samples with a gamma counter (Gammatic, C.G.R.). The intestinal clearance of chromium ([^{51}Cr] Cl) was determined in milliliters of serum per day by the ratio of radioactive mean fecal loss per day to the mean serum radioactivity.

In vitro studies. Determination and in vitro stability of α_1-AT concentration in gastric and duodenal juice and feces were also studied. (a) *Gastric juice:* α_1-Antitrypsin concentrations were determined in gastric juice from 18 subjects with different pH collected during gastric tests carried out on normal and pathologic subjects. In a second set of experiments, 1.5 mg of α_1-AT was added to 3 ml of gastric juice (n = 11) of pH < 3 and α_1-AT determinations were performed after an incubation time of 1 h at 37°C. (b) *Duodenal juice:* Duodenal juice was collected on 10 normal subjects during pancreatic function tests (secretine—CCK test) and α_1-AT determinations were performed before and after an incubation time of 2 h at 37°C. (c) *Feces:* α_1-Antitrypsin was assayed before and after an incubation period of 3 days at 37°C.

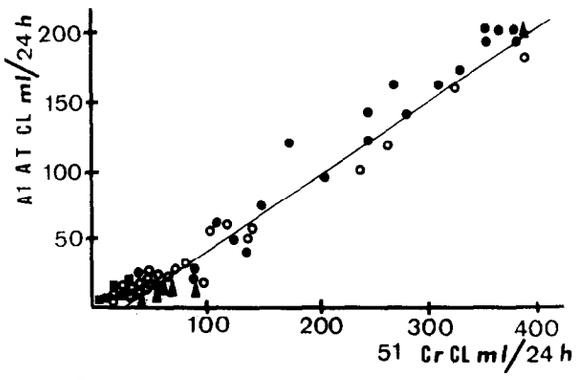

Figure 1. Correlation between α_1-AT fecal clearance and protein loss measured by [^{51}Cr]Cl_3 (r = 0.96; n = 55; p = 0.001). (●), Inflammatory bowel diseases (n = 21). (○), Noninflammatory bowel diseases (n = 23). (▲), Miscellaneous (n = 6). (■), Controls (n = 5).

Results

In Vivo Studies

There is a highly significant correlation between α_1-AT clearance and ^{51}Cr-protein clearance as shown in Figure 1 (r = 0.96; p < 0.001); α_1-AT Cl =

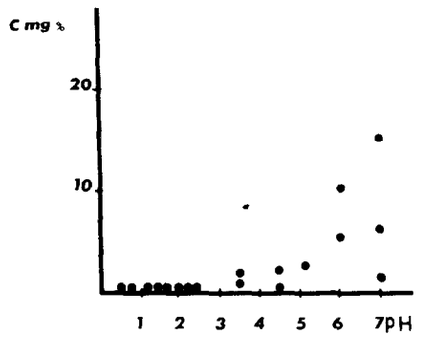

Figure 2. α_1-Antitrypsin concentration in gastric juice (n = 18).

0.55 ([^{51}Cr]Cl)—12.9 ml/day. This correlation is identical for inflammatory or noninflammatory bowel diseases. The correlation depends neither on serum α_1-AT concentration nor on stool weight. In an attempt to study the influence of the site or length of the lesions on this correlation, we showed that there was no difference between small bowel and colonic diseases. Thus α_1-AT clearance and ^{51}Cr-protein clearance behave in the same manner in these different diseases.

The upper limit of the normal for α_1-AT clearance has been previously determined in 200 healthy controls (unpublished data). It is 13 ml/day (mean + 3 SD). The upper limit of the normal for ^{51}Cr-losses is 40 ml/day (mean + 3 SD). Assuming that the values for the chromium chloride test are correct we performed a Baysian analysis between α_1-AT Cl and [^{51}Cr]Cl. The sensitivity of the test is 93.3%, the specificity is 90%, the positive predictive value of the test is 97.7%, and the negative predictive value is 75%.

Baysian Table

[^{51}Cr]Cl \ α_1-AT	>13 ml/24 h	<13 ml/24 h
> 40 ml/24 h	42	3
< 40 ml/24 h	1	9

The confidence limits on predicted mean α_1-AT Cl values are: [^{51} Cr]Cl = 100 ml/day and α_1-AT Cl = 42.11 ± 11.18 ml/day. The estimated standard deviation from regression is 39.58. The standard deviations of points about the fitted line and standard errors of slope and intercept are:

95% confidence limits on slope: $a = 0.55 \pm 0.0436$; and 95% confidence limits on intercept: $b = 12.9 \pm 8.3$ ml/day

In Vitro Studies

Gastric juice. In vivo we were unable to detect α_1-AT in gastric juice of pH below 3 (n = 18) (Figure 2). In vitro studies (n = 11) confirmed that α_1-AT is almost completely destroyed (97.2%) during incubation with gastric juice pH < 3 and 37°C for 1 h.

Duodenal juice. Incubation of duodenal juices for 2 h at 37°C show a small, but significant decrease (3%) ($p < 0.01$) of the α_1-AT concentration (Figure 3).

Feces. There is a slight decrease (2.5%/day) of α_1-AT concentration when the stools are incubated at 37°C for 24 h. After 3 days of incubation the recovery rate is 92%.

Discussion

The data presented in this paper show that the intestinal clearance of α_1-AT is a sensitive method for detecting protein-losing enteropathy. There is a highly significant correlation between α_1-AT clearance and radiolabeled protein losses expressed as clearance. It confirms our preliminary report (2) on the discriminating value of this test in protein-losing enteropathies even in children (10)

The absence of correlation difference between small intestine and colon diseases indicates that this test may be used for every site of protein-losing enteropathy.

But our in vitro results that α_1-AT is undetectable in gastric juice at pH below 3 suggest that the test is useless in exudative gastropathy. In the same way it may be noticed that the intercept of the regression curve indicates [^{51}Cr]Cl : +23.5 ml when α_1-AT Cl is 0. This finding could be explained by a degradation of a constant amount of α_1-AT in the gastrointestinal tract. This destroyed α_1-AT could be the amount delivered in the normal stomach as far as the gastric

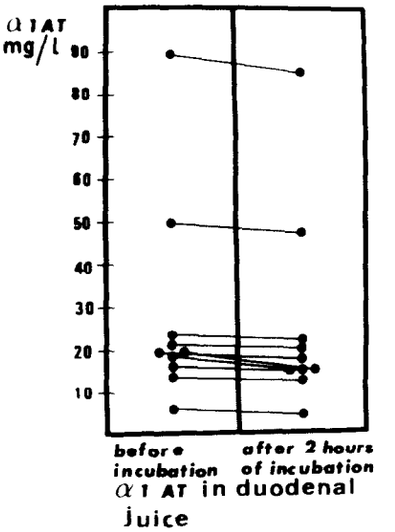

Figure 3. α_1-Antitrypsin concentration in duodenal juice before and after 2 h incubation at 37°C.

clearance of albumin has been estimated in normal subjects to 46 ml/day (3), 48–72 ml/day (12). The slope of the regression curve is 0.55. It should theoretically be 1, since the two proteins are lost by passive diffusion and have similar weight (6–8). One of the explanations of this discrepancy could be that a degradation of α_1-AT occurs during its transit into the gastrointestinal tract. We were unable to show important degradation of α_1-AT during incubation of duodenal juice and feces, but literature data (9–11) suggest that degradation might occur in the jejunum, ileum, and colon. The small destruction of α_1-AT in feces at 37°C indicates that it is unnecessary to store the stools at −20°C and they may be stored at +4°C. α_1-Antitrypsin clearance appears to be a reliable clinical test in protein loss distal to the pylorus, inflammatory bowel diseases with ulcerations, such as Crohn's disease or ulcerative colitis, as well as in diseases with disorders of intestinal lymphatics (1), cardiac failure, or in diseases with demonstrable pathology but in which the mechanism of loss is not obvious.

Conclusion

The α_1-AT Cl is able to detect protein-losing enteropathy. Its advantage is that it does not need any radioactive product and may be done in outpatients. Its low cost makes it a screening test easy to be used. As it discards the ethical problems due to radioactive products, it permits the use of protein-loss research to other fields of gastroenterology, for example, intestinal toxicity of drugs.

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