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Serum proteolytic activities and antiproteases in human colorectal carcinoma

J. A. Amiguet[1], J. Jiménez[2], J. I. Monreal[3], M. J. Hernández[1], G. López-Vivanco[5], J. R. Vidán[2], F. Conchillo[4] and P. Liso[4]

[1]Departamento de Medicina Interna, Hospital Clínico Universitario, Zaragoza; [2]Servicio Digestivo, Hospital de Navarra, Pamplona; Servicios de [3]Bioquímica y de [4]Digestivo, Clínica Universitaria de Navarra, Pamplona and [5]Serv. Oncología Médica, Hospital de Cruces, Baracaldo (Spain).

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Some proteolytic enzymes, trypsin, cathepsin B, cathepsin D, collagenase, elastase and their inhibitors, API and AMG, in serum of patients with colorectal carcinoma have been evaluated. Twenty patients belonged to stage B of colorectal carcinoma, twenty two patients to stage D (Astler and Coller classification) and a control group of thirty healthy volunteers were evaluated. Except in cathepsin D, patients exhibit higher enzymatic activities than healthy subjects, and both groups have all the proteolytic activities assayed in serum. Patients with disseminated disease have increased cathepsin B and collagenase levels, with a decrease of trypsin activity, showing an increment in API and AMG in sera. However, only the API values were significantly higher in patients with metastases. The coexistence of proteolytic activities in human sera together with their inhibitors is considered as well as the origin of these, tumoral and/or reactive, increments. Cathepsin B levels are raised in colorectal neoplasms and contribute to the destruction of the extracellular matrix and the proliferation of tumoral cells. There is evidence that a relation between collagenase like activity and tumor invasiveness exists. Cathepsin B and collagenase increases agree with the tumoral mass. On the other hand, trypsin decrease in metastatic carcinoma is probably related to the increment of their inhibitors, API and AMG, acute phase reactant proteins.

Key words: Protease, Antiprotease, Acute phase reactants, Collagenase, Cathepsin B.

Correspondence to J. I. Monreal (Tel.: 948 22 54 00-1103; Fax 948 17 22 94).

There has been an increasing interest on proteolytic enzymes and their inhibitors, both in tissues and biological fluids (3, 34). It is known how malignant tumoral cells release proteases to penetrate extracellular structures (39) in colon carcinoma (37) among other malignancies. The existence of substances in human serum able to inhibit these enzymes, such as Alpha 1 proteinase inhibitor (API) and Alpha 2 macroglobulin (AMG), both acute phase reactant proteins (APRPs) (23) has also been well documented.

The presence of cathepsin-like activity in healthy human sera together with normal API and AMG values has been described as well as how API was modified in the sera of patients with breast carcinoma (27).

The aim of the present study, encouraged by the present interest in this subject and its possible significance in physiology and human clinics, is to evaluate the possible modification of trypsin, cathepsin B, cathepsin D, collagenase, elastase, API and AMG in the serum of patients with colorectal carcinoma.

Materials and Methods

Forty two patients (31 men and 11 women, from 41 to 83 years of age) affected by colorectal carcinoma were studied. Patients with colorectal cancer and any other concomitant pathology were not selected for the study. Tumor location was rectum in 14 cases (33.3 %), sigmoid colon in 16 (38.1 %), descending colon in 7 (16.6 %), transverse colon in 3 (7.1 %), ascending colon in 1 (2.3 %) and cecum in 1 (2.3 %). According to the Astler and Coller classification, 20 patients belonged to stage B (tumor confined to the gut wall) and 22 patients to stage D (distant metastases), while a control group of 30 healthy volunteers (20 men and 10 women from 35 to 55 years of age) were also evaluated.

The sera were obtained by spontaneous coagulation at room temperature and stored at –40 °C for no longer than four months and they were defrozen only once before measurement was taken.

To measure trypsin-like activity, N-α-benzoil-L-arginina-paranitroanilide (L-BAPNA) were used as a substratum (pH 8) (42), cathepsin B with the same substratum (pH 6) (5) and cathepsin D with hemoglobin (4). Collagenase activity was determined with ^{3}H-collagen as substrate (New England Nuclear), while elastase was by immunoassay (Merck). API and AMG were measured by single radial immunodiffusion using specific antisera from Behringwerke Laboratories (30). Data were statistically analyzed using multiple mean comparison tests and Student *t* test.

Results and Discussion

Proteases.– Some of the four types of serum endopeptidases, classified according to their active components in serinproteases (trypsin, elastase), cisteinproteases (cathepsin B), asparticoproteases (cathepsin D) and metalloproteases (collagenase) (6), as well as their inhibitors, API and AMG, both APRPs have been studied. The former inhibits serinproteases (41) whereas AMG inhibits the four groups of enzymes (7).

Five proteolytic activities coexist in healthy human sera with their inhibitors, which suggests the existence of a protease/antiprotease dynamic balance, as it happens in other biological functions such as coagulation. Table I shows the serum proteolitic values in the healthy subjects and patients. All values, except cathepsin D, increase. Cathepsin B and collagenase increases were higher in the presence of a metastatic disease, whereas trypsin behaviour showed just the opposite effect. Cathepsin D activity is present in healthy

Table I. *Seric tryptic like, cathepsin B, collagenase, cathepsin D activities and elastase, API and AMG concentrations in patients with local and diseminated disease and control subjects.* Different letters express statistical differences ($p < 0.05$).

	Control (n=30)	Local (n=20)	Diseminated (n=22)
Elastase (ng/dL)	196.5 ± 19.2[a]	385 ± 50.7[b]	569.3 ± 95.1[c]
Tripsin (U/L)	1.01± 0.13[a]	9.50 ± 0.89[b]	5.34 ± 0.77[c]
Cathepsin B (U/L)	1.88 ± 0.12[a]	3.05 ± 0.33[b]	11.07 ± 1.17[c]
Collagenase (ng/dL)	19.7 ± 0.73[a]	22.8 ± 0.85[b]	26.1 ± 0.67[c]
Cathepsin D (U/L)	32.8 ± 2.21	29.5 ± 1.88	34.5 ± 1.96
API (mg/dL)	179.8 ± 8.63[a]	278.5 ± 17.0[b]	337.1 ± 19.2[c]
AMG (mg/dL)	197± 9.8[a]	274.1 ± 7.9[b]	288.3 ± 6.8[b]

sera but no modifications are seen in tumoral patients.

Increase of cathepsin B activity in women with malignancies (36) has been described as well as cathepsin D activity in breast carcinoma (27), elastase in benign ovarian tumors (33), leukemias (40) and collagenase in colon and breast carcinomas (44).

The origin of this increment could be tumoral or reactive. In the process of cancerogenesis, genetic alterations may lead to an increase in proteases synthesis by differentiated cells to facilitate tumoral invasion by extracellular matrix digestion (25). It is therefore possible that by acting on patient vascular endothelium or subsequently on tumoral neovascularization (38), these enzymes reach systemic circulation and raise their seric levels. This neoplastic origin is abundantly supported in articles reporting an increase of the proteolytic activities in the tumoral tissue and, in some cases, of their inhibitors (35). A tissular increase in trypsin like activity, its tumor associated inhibitor (TATI) (19), metalloproteinases, its tissular inhibitor (TIMP-1) (29) and cathepsin B-like activity (9, 10) has been demonstrated. They stimulate tumoral growth and invasiveness (15, 22).

Cathepsin B and collagenase increase correlates with tumoral mass. Only trypsin like activity decreases in the group with metastases, according to the presence of a remarkable increment in systemic and local inhibitors, as API.

As the reactive origin of proteolytic activities, the tumoral focus might be considered. The neoplastic mass is surrounded and infiltrated by macrophages, PMN and lymphocytes that release their proteolytic enzymes including elastase with a lytic function against the tumor (2) and the extracellular matrix (13). Something similar may occur with cathepsin B and collagenase activities, also released by PMN. Some cathepsins may also be released locally by mononuclear macrophages (8) and platelets (23), particularly if the tumor is surrounded by fibrin (11).

APRPs.– There is no complete agreement about the functions of these plasmatic proteins. They can modulate immunitary responses (24) and local inflammatory reaction due to their antiprotease and antioxidative properties (21). Since albumin levels fall while these APRPs remain high, even in the presence of metastases and a severely affected metabolism, it may be suggested that their functions must be of significant relevance for the organism. In this sense, the use of the term "acute phase response" may not be adequate in carcinomas and other chronic entities. It

might be more suitable to talk about an "activity phase response" in both acute and chronic diseases, referring to the increase in seric APRPs (18).

Activated mononuclear macrophages and neoplastic cells can release cytokines such as IL-1 or TNF which stimulate cathepsin B and collagenase synthesis and secretion (14, 17). These cytokines with IL-6, released by mononuclear macrophages after the injury induce hepatic synthesis of APRPs whereas albumin production falls to nearly 50 % (12, 31). The possible role of different hormones (21), other cytokines, certain substances related with cellular proliferation like oncostatin M (28) and proteases themselves (20) to regulate this hepatic response is now a matter for investigation. In metastatic patients, API shows higher values (table I), but this fact does not happen with AMG. This finding can be understood if we consider that the mean life for the complex protease-AMG is estimated at eight minutes, while that of AMG is approximately ten days (16). Uptake is, therefore, much higher for AMG than for API. Besides, AMG can inhibit the four types of proteinases and can accept proteolytic enzymes previously transported by API (32), which means a higher uptake of this protein.

In malignant tumoral disease and particularly in colorectal carcinoma, there is probably, a kind of coexistence of APRPs with reactive proteolytic enzymes but at a higher dynamic level of uncertain significance. The increase in this equilibrium can be measured in serum with APRPs as a valid biological follow-up and prognostic factor for tumoral patients (1, 26, 43).

In further studies, the isolation and characterization of tumoral proteases will become fundamental, in the search for new diagnostic parameters in the long process of cancerogenesis but in the initial stages of malignancy.

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Se valora la posible modificación de algunas proteasas (tripsina, catepsina B, catepsina D, colagenasa y elastasa, y sus inhibidores, API y AMG, en suero de pacientes con carcinoma colorrectal. Se estudian 20 pacientes con carcinoma en estadio B y 22 en estadio D de la clasificación de Astler y Coller, así como un grupo de 30 sujetos sanos. Todas las proteasas, excepto la catepsina D, muestran valores más elevados en los pacientes que en los controles, aunque en ambos grupos se presentan en suero todas las enzimas ensayadas. Los pacientes con enfermedad diseminada alcanzan niveles más elevados de catepsina B y colagenasa, y menor actividad de tipo tripsina. Se discute el sentido de la coexistencia en suero de actividades proteolíticas y de sus inhibidores, así como el posible origen de este incremento, tumoral y/o reactivo. La catepsina B se eleva en neoplasias y actúa destruyendo la matriz extracelular y potenciando la proliferación de células tumorales. Hay también evidencia de una relación entre la actividad colagenasa y la progresión tumoral. La actividad de ambas enzimas se eleva en relación a la masa tumoral. Por otra parte, el descenso de la tripsina en el carcinoma metastatizado probablemente guarda relación con el incremento de sus inhibidores, API y AMG, proteínas reactantes de fase aguda.

Palabras clave: Proteasa, Antiproteasa, Reactantes de fase aguda, Colagenasa, Catepsina B.

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