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Pancreatic Cancer as a Model: Inflammatory Mediators, Acute-phase Response, and Cancer Cachexia

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Abstract. Patients with pancreatic cancer frequently develop the syndrome of cancer cachexia. Pro-inflammatory cytokines have been strongly implicated in the pathogenesis of this syndrome. In patients with pancreatic cancer an acute-phase response (an index of pro-inflammatory cytokine activity) is associated with accelerated weight loss, hypermetabolism, anorexia, and a shortened duration of survival. However, little is known about the primary significance of the acute-phase response in terms of altered hepatic export protein synthesis rates and its potential impact on the body's nitrogen economy. In a recent series of studies on weight-losing pancreatic cancer patients with hypoalbuminemia we have demonstrated albumin synthesis to be unaltered whereas fibrinogen synthesis is increased two- to threefold compared with healthy controls. Because of the mismatch in amino acid composition between the body's main labile amino acid reserve (skeletal muscle) and that of acute-phase proteins, these results lend support to the concept that in pancreatic cancer the reprioritization of body protein metabolism during an acute-phase response may well be a significant factor in the loss of lean tissue in these patients.

Most patients with advanced cancer lose weight, and a proportion develop the syndrome of cancer cachexia, a major source of morbidity and mortality [1, 2]. Among patients with solid epithelial malignancies, those with unresectable pancreatic cancer have perhaps the highest incidence of cachexia [3] with more than 80% losing weight by the time of diagnosis [4]. The median duration of survival of pancreatic cancer patients is somewhere between 3 and 6 months. Following the relief of obstructive jaundice with either an endobiliary stent or surgical bypass, and in the absence of an effective form of systemic antineoplastic therapy, one of the major areas whereby optimal palliation might be achieved would be the prevention or reversal of weight loss in such patients.

It might be supposed that the best way to treat weight loss in cancer would be simply to increase the patient's food intake. However, patients frequently suffer from anorexia or early satiety, and the more invasive forms of nutritional support such as enteral tube feeding or parental nutrition are often inappropriate within the palliative care setting. Furthermore, there appears to be a partial block to the accretion of lean tissue following administration of conventional nutritional support [5], which has led investigators to try to understand the basic pathophysiology of weight loss in cancer in order to develop more specific and effective nutritional support strategies [6].

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Pro-inflammatory Cytokines and Acute-phase Response

Over the past 10 to 15 years it has become evident that pro-inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF), interleukin-6 (IL-6), and interferon gamma can all induce various features of the cachexia syndrome in animal models [7]. Moreover, antagonism of such cytokines in tumor-bearing animal models of cachexia can attenuate both the anorexia and a variety of the metabolic changes associated with weight loss [7]. It is not clear what might be the role of such cytokines in human cancer cachexia.

With major differences between various cytokine bioassays and immunoassays, interference from the presence of a variety of binding proteins and soluble receptors and the fact that most cytokines act in a local rather than a systemic manner, trying to relate serum cytokine concentrations to metabolic change is fraught with difficulty. In contrast, acute-phase proteins are readily measured in the circulation. The latter are a group of plasma proteins whose concentration changes after an inflammatory stimulus. Those that increase are known as positive acute-phase reactants (e.g., C-reactive protein or fibrinogen), whereas those that decrease are known as negative acute-phase proteins (e.g., albumin or transferrin). The positive acute-phase proteins fulfill a variety of functions but principally are thought to help in the body's response to infection and may also have a role in healing and repair. In the human hepatocyte the synthesis of acute-phase reactants is thought to be strongly influenced by pro-inflammatory cytokines such as IL-6 but also TNF and IL-1 [8]. We have therefore selected the presence of an elevated circulating concentration of C-reactive protein (CRP) as an index of pro-inflammatory cytokine activity and have used this index to

investigate the potential role of pro-inflammatory cytokines in various aspects of the cachexia syndrome.

We have confirmed that CRP is indeed a valid index for tissue pro-inflammatory cytokine activity [9]. Peripheral blood mononuclear cells were used as a surrogate for tissue macrophages. Cells were isolated from pancreatic cancer patients and compared with healthy controls. Production rates of IL-6 and TNF correlated closely with the presence or absence of an acute-phase response.

Acute-phase Response and Energy Balance

The prevalence of an acute-phase response (defined as a CRP concentration of more than 10 mg/L) at or near the time of diagnosis of unresectable pancreatic cancer is about 40%. As patients progress with their illness, the prevalence rises to approximately 80% at or near the time of death. During this time the mean weight loss increases from approximately 10% of preillness stable weight to more than 20% [10]. Thus during disease progression the presence of an acute-phase response parallels increasing severity of weight loss.

The development of a negative energy balance and weight loss can be accounted for by a reduction in food intake, an increase in energy expenditure, or a combination of the two. We have examined the relation between an acute-phase response and both sides of the energy balance equation. In patients with pancreatic cancer, resting energy expenditure is generally increased when compared with that of healthy controls [9, 11]. Most interesting, however, is the observation that the presence of an acute-phase response is associated with the greatest degree of hypermetabolism [9]. Similarly, if one examines the energy intake of a group of pancreatic cancer patients, the presence of an acute-phase response is associated with a significant reduction in energy intake (accounting for a calorie deficit of approximately 500 calories per day) [11]. Pro-inflammatory cytokine activity therefore appears to be associated not only with altered host energy metabolism but also with reduced appetite, thereby contributing to the accelerated weight loss observed with cachexia.

The significance of an acute-phase response is further emphasized by the observation that the median survival from the diagnosis of unresectable pancreatic cancer in patients with an acute-phase response is approximately 70 days compared with 220 days in those with no response [10]. When all known prognostic variables are entered into a multivariate analysis and each factor is adjusted for the influence of the other, the presence of an acute-phase protein response is the single most significant independent predictor of a shortened survival duration in these patients [10].

Acute-phase Response as a Primary Mechanism of Wasting

It has long been recognized that with the onset of inflammation there is a reprioritization of body protein metabolism with increased circulating levels of positive acute-phase proteins and the loss of body protein at the level of skeletal muscle. Following the discovery that pro-inflammatory cytokines such as IL-6 were key mediators of the acute-phase response in the liver [8], it was also suggested that pro-inflammatory cytokines might act directly at the level of skeletal muscle to provide amino acid precursors for acute-phase protein synthesis. However, although initial experiments with crude monocyte supernatants suggested that cytokines might be implicated directly in accelerating skeletal muscle protein breakdown, the use of recombinant cytokines such as IL-1 or TNF failed to confirm a direct action at the level of skeletal muscle [12]. The recent characterisation of a novel cancer cachectic factor [13] has, however, once again raised the possibility that there may be circulating factors able to directly induce skeletal muscle protein breakdown in the weight-losing cancer patient [14].

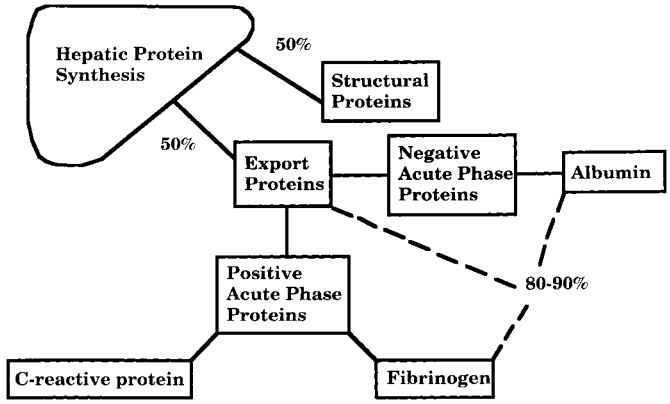

Fig. 1. Nitrogen economy of the liver and the importance of albumin and fibrinogen synthesis in export protein synthesis.

Independent of the mechanism whereby amino acids are mobilized from skeletal muscle, a key question concerns the degree to which the body's overall protein metabolism is altered by the presence of an acute-phase response. These alterations in turn determine the overall significance of the acute-phase response in relation to the loss of lean tissue. We have therefore been particularly interested in measuring the synthesis rates of hepatic export and structural proteins in patients with cancer cachexia.

It is thought that approximately 50% of protein synthesized in the liver is directed toward structural proteins and the other 50% toward export proteins (Fig. 1). In a previous study in patients with colon cancer (all with an ongoing acute-phase response and concomitant weight loss), we demonstrated that structural protein synthesis is reduced by some 30% when compared with that in healthy controls [15]. This reduction might represent a form of intrahepatic nitrogen economy whereby, in the face of increased demand for amino acids for export protein synthesis, turnover is reduced in the structural protein compartment. Alternatively, such a finding may indicate a deterioration in overall liver function and reduced capacity for metabolic regulation.

Our recent studies have been directed toward quantifying the alterations in synthesis rates of positive and negative acute-phase proteins in patients with pancreatic cancer cachexia. As indicated previously, the definition of a positive acute-phase protein is one whose plasma concentration rises during an inflammatory response whereas that of a negative reactant falls. The concentration of a plasma protein is determined in part by the relation between its rate of synthesis and degradation (Fig. 2). Previous studies in isolated human hepatocytes have suggested that one of the major mechanisms whereby the concentration of a positive acute-phase protein increases when exposed to IL-6 is that there is a massive increase in its synthesis rate [8]. Similarly, it has been suggested that the fall in the concentration of negative acute-phase proteins might be principally accounted for by a reduction

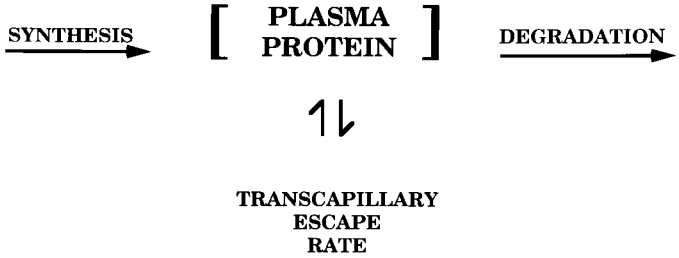

Fig. 2. Determinants of a plasma protein's concentration.

in synthesis rate [8]. The latter observation has not only been suggested by in vitro studies but also from in vivo studies [16]. A plasma protein's concentration is also influenced by the rate at which it leaves the intravascular compartment (transcapillary escape rate, or TER), and it is known that pro-inflammatory cytokines such as IL-6 can directly increase endothelial permeability [17]. In vivo studies have been somewhat controversial. Some have suggested a markedly increased TER for albumin in cachectic cancer patients [18], whereas other investigators, although confirming an elevated TER, have found no correlation between albumin concentration and TER [19]. Given this complex situation, to evaluate the metabolic significance of an acute-phase response in the loss of body protein associated with cancer cachexia it is necessary to measure directly hepatic export synthesis rates in vivo.

In terms of hepatic export protein synthesis, albumin is the major negative acute-phase protein and perhaps accounts for 50% to 70% of total liver export protein synthesis. In terms of positive acute-phase proteins, although CRP can be regarded as the archetypal positive acute-phase reactant it is measured in only milligram quantities in the plasma. Hence proteins such as fibrinogen (which are measured in gram quantities) are more significant in terms of the liver's nitrogen economy. Taken together, albumin and fibrinogen are thought to account for approximately 80% of the liver's output in normal individuals. We have therefore studied the kinetics of albumin and fibrinogen in pancreatic cancer patients with an ongoing acute-phase response and weight loss.

Albumin and Fibrinogen Synthesis Rates in Cachectic Pancreatic Cancer Patients

Patients were studied in the fasting state. Fractional synthesis rates were estimated from the rate of incorporation of deuterated phenylalanine into proteins during a flooding dose protocol [20]. Results are summarized in Figures 3 and 4. The cancer patients had lost approximately 18% of their preillness stable weight and were hypoalbuminemic with elevated CRP and fibrinogen levels at the time of the study. Their intravascular albumin mass was reduced, although the albumin fractional synthesis rate was significantly increased. Thus, although the albumin pool was turning over faster owing to the fact that the pool size had decreased, the total albumin synthesis rate (approximately 12 g/day) was unchanged compared with that of healthy controls [21]. These findings suggest that, contrary to expectation, during an acute-phase response in cancer patients the primary reason for a reduced albumin concentration is not a reduction in synthesis rate. Degradation, TER, or both must be increased.

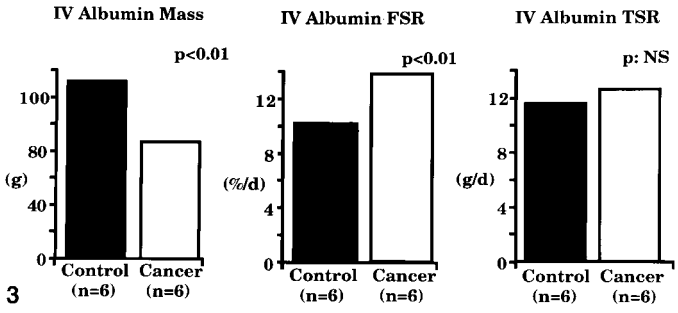

Fig. 3. Albumin kinetics in weight-losing pancreatic cancer patients (n = 6) and healthy controls (n = 6). IV: intravenous; FSR: fractional synthetic rate; TSR: total synthetic rate.

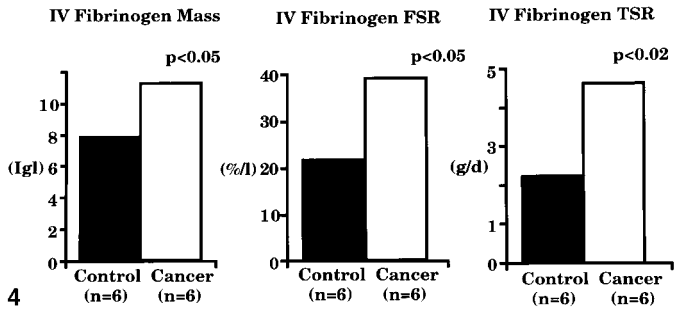

Fig. 4. Fibrinogen kinetics in weight-losing pancreatic cancer patients (n = 6) and health controls (n = 6).

In contrast to albumin, fibrinogen concentration and intravascular mass was significantly elevated in the pancreatic cancer patients. The fractional synthesis rate was markedly increased, and so when the total fibinogen synthesis rate was calculated (approximately 5 g/day), the values were 200% to 300% higher than that of controls [22].

It therefore appears that in wasted cancer patients with an ongoing acute-phase response albumin synthesis continues unabated, and fibrinogen synthesis is greatly increased. These measurements were undertaken in the fasting state, and clearly the changes may be even more marked during the fed state. Moreover, fibinogen is only one of a whole range of acute-phase reactants, and therefore the total burden of acute-phase protein synthesis on the liver may be substantial. It has been suggested that in the semistarving state, because of the differences in the amino acid composition of skeletal muscle and acute-phase proteins, proportionally more skeletal muscle must be broken down to synthesize acute-phase proteins [23]. Furthermore, should the level of inflammation vary (e.g., in pancreatic cancer patients when they experience an episode of cholangitis), the potential inefficiency in the system would exaggerate the overall net loss of body protein. Thus the level of altered hepatic export protein synthesis observed in cachectic cancer patients may well have a significant impact on whole-body nitrogen economy and could contribute to the loss of lean tissue.

Conclusions

Pancreatic cancer appears to be a useful model for the study of cancer cachexia. Most of these patients develop severe wasting, and it seems that pro-inflammatory cytokine-mediated metabolic

events, such as the acute-phase response, are associated with altered energy and altered protein metabolism of these patients. Such findings imply that down-regulation of pro-inflammatory cytokines and the acute-phase response should be a valid target for therapeutic intervention, the subject of our current studies [24, 25].

Résumé

Le syndrome de cachexie des cancéreux est fréquent chez le patient ayant un cancer du pancréas. Les cytokines pro-inflammatoires ont été fortement impliqués dans la pathogenèse de ce syndrome. Chez les patients ayant un cancer pancréatique la réponse de la phase aiguë (indexe d'activité des cytokines pro-inflammatoires) est souvent associée à une perte de poids importante, un hypermétabolisme, une anorexie et un raccourcissement de la survie. La signification de cette réponse, en termes d'altération des taux de synthèse protidique extra-hépatique et son impact potentiel sur l'économie de l'azote du corps, n'est, par contre, pas bien élucidé. Dans une série récente de patients atteints de cancer du pancréas, ayant perdu du poids avec hypoalbuminémie, nous avons démontré que la synthèse d'albumine était normale alors que la synthèse de fibrine était augmentée 2 à 3 fois celle des contrôles sains. En raison de la disparité dans la composition entre la réserve corporelle principale en acides aminés labiles (muscle du squelette) et celle des protéines de la phase aiguë, ces résultats tendent à prouver que dans le cancer du pancréas, la perturbation de la prioritisation du métabolisme des protéines pendant la réponse de la phase aiguë pourrait bien être un facteur significatif dans la perte de tissu maigre chez ces patients.

Resumen

Los pacientes con cáncer del páncreas generalmente desarrollan el síndrome de la caquexia del cáncer. Las citocinas proinflamatorias han sido fuertemente inculpadas en la patogénesis del síndrome. En los pacientes con cáncer pancreático se asocia una fase de respuesta aguda (que es indicativa de actividad proinflamatoria citocínica) con pérdida rápida de peso, hipermetabolismo, anorexia y reducción en el período de supervivencia. Sin embargo, poco se sabe acerca del significado de la fase de respuesta aguda en términos de alteración de la síntesis hepática de proteínas de exportación y su impacto potencial sobre la economía corporal del nitrógeno. En una reciente serie de estudios sobre pacientes con cáncer pancreático que exhibían pérdida de peso e hipoalbuminemia, hemos podido demostrar que no hay alteración en la síntesis de albúmina, en tanto que se observa una elevación de 2–3 veces en la síntesis de fibrinógeno, en comparación con individuos sanos. Debido a la discrepancia en la composición de aminoácidos entre las reservas corporales principales de aminoácidos lábiles (músculo esquelético) y las proteínas de fase aguda, nuestros resultados dan soporte al concepto de que el paciente con cáncer del páncreas, la reprioriización del metabolismo proteico corporal en el curso de la respuesta de fase aguda puede ser un factor de significación en la pérdida de tejido magro.

Acknowledgments

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