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The relationship between plasma cholesterol, amino acids and acute phase proteins in sepsis*

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Summary. The purpose of the study was to correlate degree of hypocholesterolemia to changes in plasma levels of amino acids and other metabolic variables in severely injured septic patients. Measurements included plasma cholesterol, full amino-acidograms, acute phase proteins, complementary variables and blood cell counts. The Fischer plasma molar amino acid ratio (leucine + isoleucine + valine)/(phenylalanine + tyrosine) was calculated. Plasma cholesterol for all measurements ($n = 145$) was 3.1 ± 1.1 mmol/L and, upon entry in the study, it was correlated inversely with sepsis severity score ($p < 0.05$). Along the clinical course, changes in cholesterol were clearly paralleled by opposite changes in C-reactive protein, which was the best correlate of cholesterol ($r^2 = 0.70$, $p < 0.0001$). Furthermore cholesterol was inversely related to phenylalanine, fibrinogen, lactate and white blood cell count, and directly to the Fischer molar amino acid ratio, cystathionine, methionine, glycine and transferrin (r^2 between 0.36 and 0.15, $p < 0.0001$ for all). Within this pattern of correlations, cholesterol was also directly related to alkaline phosphatase, which accounted for the effect of cholestasis, when present. For any given value of the other variables, cholesterol increased significantly with increase in alkaline phosphatase ($p < 0.0001$). C-reactive protein (CRP, mg/dl) and alkaline phosphatase (ALKPH, U/L) together in the same regression explained 79% of the variability of cholesterol (CHOL, mmol/L): $CHOL = 5.90–0.74[Log_eCRP] + 0.004[ALKPH]$; multiple $r^2 = 0.79$, $p < 0.0001$. Inclusion in this regression of other variables did not increase the r^2. By using only amino acid variables, the best fit was provided by a regression including the Fischer ratio and cystathionine, which explained 55% of the variability of cholesterol (multiple $r^2 = 0.55$ $p < 0.0001$), and this result was not improved by the inclusion of other amino acids. These data show that severity of hypocholesterolemia in sepsis is quantifiably related to changes in plasma amino acids, and to severity of acute phase response and metabolic decompensation. More study is needed to understand whether hypocholesterolemia in sepsis has only diagnostic or prognostic implications, or that it may also contribute actively to worsening of the disease.

Keywords: Amino acids – Plasma cholesterol – Acute phase proteins – C-reactive protein – Sepsis

Introduction

In critically ill and surgical patients hypocholesterolemia has recently gained importance as an index of severity of illness and a predictor of poor prognosis. In these patients several mechanisms determining hypocholesterolemia may coexist and act cumulatively to induce extremely low cholesterol levels (Fraunberger et al., 1998 and 1999; Giovannini et al., 1999 and 2003). In different diseased states the involved mechanisms may include abnormal hepatic lipoprotein synthesis, abnormal lipoprotein receptor activity and hemodilution from blood loss. Sepsis is one of such diseases and, although occurrence of hypocholesterolemia has been related to an effect of increased cytokine levels on plasma lipoprotein pattern, it is not clear whether degree of hypocholesterolemia is quantifiably related to severity of inflammatory response and septic metabolic decompensation. We have assessed this aspect in a prospective study, by correlating changes in plasma cholesterol with changes in plasma amino acids, acute phase proteins and other metabolic variables in a group of patients with post-traumatic sepsis.

Materials and methods

The study was performed on eight severely injured trauma patients who developed sepsis. Age (mean ± SD) was 32 ± 19 yrs, weight 76 ± 13 Kg, height 175 ± 6 cm, injury severity score 30 ± 14 (Greenspan et al., 1985). The patients had a combination of abdominal, chest and head injuries, and cause of sepsis was intraabdominal, pulmonary or extensive soft tissue infection. Diagnosis of sepsis was based on simultaneous occurrence of a temperature >38.3°C, white blood cell count $>12 \times 10^9$/L and clear evidence of infection confirmed by positive cultures from blood, surgical

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drainage of infected areas or sputum in the case of pulmonary sepsis. Sepsis severity score (Skau, 1985) upon diagnosis of sepsis and entry in the study was 28 ± 13. One patient died of progressive septic metabolic decompensation with multiple organ dysfunction syndrome, one of sudden cardiac failure and the remainder survived. All patients were undergoing total parenteral nutrition (32 ± 6 kcal/kg/day, 74% glucose and 26% fat, and 1.4 ± 0.3 g/kg/day amino acids, 50% branched-chain). The study was carried out prospectively, with informed consent from patients or relatives, and institutional approval. Measurements were performed every 8 hours, from diagnosis of sepsis until criteria for persistent sepsis were fully met. No patient had oliguric renal failure, four patients developed moderate to severe cholestasis. Mean duration of the study was 6 days per patient. Each measurement included the levels of plasma cholesterol, C-reactive protein, fibrinogen, albumin, transferrin, ceruloplasmin, alpha-1 antitrypsin, alpha-2 macroglobulin, creatinine, glucose, blood urea nitrogen, lactate, alkaline phosphatase, bilirubin, sodium, potassium, calcium, blood cell count, and full aminoacidogram. The Fischer plasma molar amino acid ratio (leucine + isoleucine + valine)/(phenylalanine + tyrosine) was also calculated to assess severity of septic metabolic dysregulation and liver dysfunction (Fischer et al., 1976; Freund et al., 1979; Jeppsson et al., 1981). Statistical analysis was based on least square regression, with analysis of residuals, skewness, kurtosis, confidence intervals for regression coefficients (by setting the level of acceptance of general regressions at $r^2 > 0.15$ and $p < 0.0001$) and a "simplest best fit" program selecting the simplest possible regressions controlling the largest possible variability of cholesterol, based on Mallows' Cp criteria (Seber, 1977). This allowed assessment of the hierarchy of correlations and the main driving factors accounting for the variability of cholesterol.

Table 1. Top: mean ± SD of main correlates of cholesterol, for cholesterol $\leq$ or >3.1 mmol/L (3.1 mmol/L = mean value for all measurements), showing tendency of lower cholesterol to be associated with higher C-reactive protein, fibrinogen, lactate, white blood cell count, phenylalanine, and with lower values of the other variables

Cholesterol, mmol/L	≤3.1 (n = 70)	>3.1 (n = 75)
C-reactive protein, mg/L	229 ± 101	74 ± 24
Fibrinogen, μmol/L	36 ± 9	30 ± 8
Lactate, μmol/L	12 ± 3	9 ± 1
White blood cells, $\times 10^9$/L	27.8 ± 7.7	18.9 ± 4.8
Transferrin, g/L	1.39 ± 0.34	1.74 ± 0.44
Fischer molar amino acid ratio	4.0 ± 1.1	5.5 ± 1.3
Phenylalanine, μmol/L	113 ± 26	82 ± 26
Cystathionine, μmol/L	4 ± 3	9 ± 4
Methionine, μmol/L	31 ± 9	36 ± 7
Glycine, μmol/L	200 ± 43	224 ± 36

Cholesterol = 5.90–0.74(Log_eCRP) + 0.004(ALKPH)
total n = 145, $r^2 = 0.79$, $p < 0.0001$ for whole regression and each independent variable

Bottom: simplest best regression on cholesterol (*CRP*, C-reactive protein; *ALKPH*, alkaline phosphatase, U/L, normal range 25–100). See text for explanations

Results

Plasma cholesterol for all measurements was 3.1 ± 1.1 mmol/L (mean ± SD; range 1.2–5.7; total n = 145). Upon entry in the study it was 2.0 ± 0.3 (range 1.4–2.4), and correlated inversely with sepsis severity score ($r^2 = 0.80$, $p < 0.05$). Cholesterol level fluctuated in individual patients during the clinical course, with a trend towards a progressive increase in survivors, as opposed to steady progressive decrease in the patient who died of septic metabolic decompensation. Changes in cholesterol in each patient were evidently paralleled by opposite changes in C-reactive protein, and were also associated with changes in other metabolic variables considered in the study. To better assess these correlations over an extended frame of reference, beyond the characteristics of individual patients, all 145 measurements were pooled and processed together. Regression analysis showed that, in all measurements pooled together, C-reactive protein was the best correlate of cholesterol ($r^2 = 0.70$, $p < 0.0001$). In addition to C-reactive protein, cholesterol was related inversely to phenylalanine, fibrinogen, lactate and white blood cell count, and directly to the Fischer molar amino acid ratio, cystathionine, methionine, glycine and transferrin (r^2 between 0.36 and 0.15, $p < 0.0001$ for all). The relationship with the Fischer amino acid ratio ($r^2 = 0.36$, $p < 0.0001$) resulted from the combination of a highly significant inverse relationship with phenylalanine ($r^2 = 0.24$, $p < 0.0001$) and weaker or not significant relationships with the branched chain amino acids and tyrosine. The tendency of cholesterol to decrease with increase in C-reactive protein, fibrinogen, phenylalanine, lactate, white blood cell count, and with the decrease in other variables, was also evident by grouping patient measurements with cholesterol $\leq$ or >3.1 mmol/L (the mean value for all measurements) (Table 1). Within the described pattern of correlations, cholesterol was also simultaneously and directly related to alkaline phosphatase, which accounted for the effect of cholestasis, when it was present. For any given value of the other variables, cholesterol increased significantly with the increase in alkaline phosphatase ($p < 0.0001$). C-reactive protein and alkaline phosphatase, together in the same regression, explained 79% of variability of cholesterol (multiple $r^2 = 0.79$, $p < 0.0001$, regression in Table 1); inclusion in this regression of any other variable considered in the study did not increase the r^2. By using only amino acid variables, the best simultaneous fit was provided by a regression including the Fischer ratio and cystathionine, which explained 55% of the variability of cholesterol (multiple $r^2 = 0.55$, $p < 0.0001$), and this result was not improved by the inclusion of other amino acids. Although the inclusion of alkaline phosphatase yielded a larger r^2 ($r^2 = 0.82$, $p < 0.0001$), the strength of this regression was

limited by the widening of confidence intervals for the cystathionine coefficient. With regard to findings excluded from the main results, it is worth mentioning that cholesterol was also related inversely to prothrombin time and directly to alpha-2 macroglobulin, ceruloplasmin and platelet count, however only at a $r^2 < 0.15$ and $p < 0.05$ level, while direct trends with the branched chain amino acids and albumin only reached borderline significance. Furthermore, the tendency of cholesterol to be related directly to tryptophan and inversely to alpha-1 antitrypsin was explained totally, in multiple regression analysis, by covariation of these variables with alkaline phosphatase, and real correlations with cholesterol could not be confirmed.

Discussion

Severity of the inflammatory response in sepsis is usually assessed by leukocytosis, although this is an inconsistent finding. It is more reliably assessed by changes in acute phase proteins, in particular by increased C-reactive protein, or also by other changes including increased fibrinogen and decreased transferrin. In turn, severity of septic metabolic decompensation may be assessed by a series of biochemical changes, which include increased plasma lactate and typical abnormalities of plasma amino acid levels, such as increased phenylalanine due to septic liver dysfunction and decreased Fischer ratio (Siegel et al., 1979 and 1982; Pittiruti et al., 1989; Freund et al., 1979; Jeppsson et al., 1981). More recently, hypocholesterolemia has also been enrolled amongst the biochemical abnormalities which characterize sepsis, however its relationship with severity of illness has not been clearly established. Our study has shown that degree of hypocholesterolemia is quantifiably related to severity of acute phase response and septic metabolic decompensation, as reflected by changes in plasma amino acids, C-reactive protein, other proteins, lactate and leukocytosis. Increase in C-reactive protein was better correlated with decrease in cholesterol than changes in any other parameter considered in the study, and accounted for 70% of the variability of cholesterol. Such a strong correlation was a remarkable finding because it was obtained in all measurements pooled together, and thus in spite of the interpatient variability and of the many additional factors which might have affected cholesterol in individual patients. In addition to increase in C-reactive protein, most of the other abnormalities correlated with hypocholesterolemia represented well-known components of the pattern of more severe septic inflammation and decompensation. Decreases in cystathionine, methionine and glycine, less commonly reported within such a pattern, are nevertheless consistent with reduced availability and/or enhanced consumption of sulphur amino acids and glycine for anti-inflammatory and anti-oxidant defense in more severe septic states (Grimble, 1994; Wheeler et al., 1999; Chiarla et al., 2000; Grotz et al., 2001). Our results, by expanding findings from previous studies, provide a wide frame of reference to correlate degree of hypocholesterolemia to degree of severity of sepsis, and also reconfirm an effect of cholestasis in increasing cholesterol, or in moderating the degree of hypocholesterolemia related to other causes (Alvarez and Ramos, 1986; Sammalkorpi et al., 1990; Bentz and Magnette, 1998; Giovannini et al., 1999 and 2003; Stachon et al., 2000). The coefficient for alkaline phosphatase in the regression in Table 1 provides a quantitative figure, for the estimate of this effect, which is close to the figures which were determined previously in other categories of patients (Giovannini et al., 1999 and 2003). The results in our study and the relationship between cholesterol and C-reactive protein are also consistent with dependency of hypocholesterolemia on enhanced production of proinflammatory reactants, through reduction of hepatic lipoprotein synthesis and/or stimulation of lipoprotein receptor activity, with a variable contribution of liver dysfunction (Fraunberger et al., 1998, 1999; Giovannini et al., 2003; Sammalkorpi et al., 1990; Akgün et al., 1998; Cerra et al., 1979; Siegel et al., 1982). More study is needed to understand whether hypocholesterolemia has only diagnostic and prognostic implications, or that it may also contribute to worsening of the disease, thus involving the need for more specific treatments (Gordon et al., 1996; Bakalar et al., 2000).

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