Changes in adrenal steroidogenesis in spontaneous bacterial peritonitis and sterile ascites

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تغيُّرات توليد الستيرويدات الكظرية في التهاب الصفاق الجرثومي التلقائي والحَبَن العقيم

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الخلاصة: قد يكون للأندروجينات الكظرية، ولاسيَّما الجهاز الديهيدروئيبي أندروستيرون، آثار تنظيمية مهمة على الجهاز المناعي في الإنسان. وقدتم في هذه الدراسة قياس التغيُّرات التي تطرأ على توليد الستيرويدات الكظرية لدى 13 مريضاً بتشمّع [تليّف] الكبد بدون عدوى مصحوب بحَبَن عقيم، و13 مريضاً بالتشمّع مصحوباً بالتهاب الصفاق الجرثومي التلقائي، وعلاقة ذلك بمستويات الانترلوكين IL-6 في الدورة الدموية، وأُجريت المقارنة مع 10 شواهد أصحاء متماثلين في السن. وتبيَّن أن وخامة التهاب الصفاق الجرثومي في حالة تشمّع الكبد تترافق بنسبة يُعْتَدُّ بها إحصائياً مع ارتفاع مستوى الانترلوكين-6 والكورتيزول في المصل، مع انخفاض الديهيدروئيبي أندروسيترون نسبةً إلى تركيزات الانترلوكين-6 في المصل، ومن ثَـمَّ فهناك حاجة إلى إجراء دراسات دقيقة وطويلة الأجل على هرمون الديهيدروئيبي أندروسيترون الذي يعطى لمرضى التشمّع، لتقييم مدى مأمونيته في تحسين عدد من الحالات المرضية التي تمثِّل مضاعفات لتشمّع الكبد.

ABSTRACT Adrenal androgens, particularly dehydroepiandrosterone (DHEA), may have important regulatory effects on the immune system in humans. This study measured the changes in adrenal steroidogenesis in 13 non-infected cirrhosis patients with sterile ascites and 13 patients with spontaneous bacterial peritonitis and the relation with circulating interleukin-6 (IL-6) levels. Comparisons were made with 10 healthy age-matched control subjects. The severity of bacterial peritonitis in liver cirrhosis was significantly associated with enhanced serum IL-6 and cortisol levels, and a decrease in serum DHEA sulfate in relation to serum IL-6 concentrations. Careful, long-term studies on DHEA administered to cirrhosis patients are needed to assess its safety in improving a number of pathological conditions that complicate liver cirrhosis.

Modification de la stéroïdogenèse surrénale dans la péritonite bactérienne spontanée et l'ascite stérile

RÉSUMÉ Les androgènes surrénaux, notamment la déhydroépiandrostérone (DHEA), peuvent avoir des effets régulateurs importants sur le système immunitaire des humains. Cette étude a mesuré les modifications de la stéroïdogenèse surrénale chez 13 patients cirrhotiques non infectés présentant une ascite stérile et 13 patients atteints de péritonite bactérienne spontanée, ainsi que la relation avec les niveaux d'interleukine 6 circulante (IL-6). Des comparaisons ont été effectuées avec 10 sujets témoins sains appariés sur l'âge. La gravité de la péritonite bactérienne dans la cirrhose hépatique était significativement associée à des niveaux accrus d'IL-6 et de cortisol sériques et à une diminution du taux sérique de sulfate de DHEA par rapport aux concentrations sériques d'IL-6. Il est nécessaire de réaliser des études approfondies et à long terme sur l'administration de DHEA aux patients cirrhotiques afin d'évaluer son innocuité et sa capacité à améliorer certains états pathologiques qui compliquent la cirrhose hépatique.

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Introduction

A variety of experimental and human studies support the interaction between the hypothalamic–pituitary–adrenal (HPA) axis and the immune system during inflammatory response [*1,2*]. Activation of the HPA axis during immune stimulation is mediated by cytokines and is manifested by increased secretion of adrenocorticotropic hormone (ACTH), which ultimately stimulates synthesis and release of glucocorticoids from the adrenal glands. Cytokines are signalling proteins mediating cell–cell communication during inflammatory and immune responses. Among the cytokines first discovered was interleukin-6 (IL-6), which is still the subject of intensive investigations because of its ubiquity and functional diversity [*3–5*]. Indeed, IL-6 appears to be a major component of the immune system, mediating interactions between the immune and neuroendocrine systems [*6*].

Inflammation is a stress condition and patients with hepatic cirrhosis are particularly susceptible to bacterial infections because of increased bacterial translocation, possibly related to liver dysfunction and reduced reticuloendothelial function. Among these infections is spontaneous bacterial peritonitis, which is directly responsible for 30%–50% of deaths of cirrhosis patients [*7*]. Chronic exposure to elevated circulating levels of cytokines in chronic hepatic inflammation may lead to HPA axis dysregulation [*8*], resulting in changes to steroidogenesis that are characterized by inadequately low cortisol secretion in relation to the systemic inflammatory response. In addition, there is a dissociation of cortisol and adrenal androgens with a marked reduction of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) relative to cortisol in various chronic inflammatory diseases [*9*]. The secretion of DHEAS is stimulated by ACTH, but there is no feedback control of DHEA secretion at the HPA axis [*10*]. The decrease in DHEAS levels is a deleterious process, particularly during chronic inflammatory diseases [*11*].

Hence, understanding changes in the HPA axis in patients with chronic liver disease may provide insight into and potentially novel therapeutic strategies for the proper management of those patients. We hypothesized that sustained elevation of IL-6 levels in patients with liver cirrhosis might be associated with dysregulation of adrenal steroidogenesis and defective adrenal DHEAS production. The current study was therefore initiated to characterize the changes in adrenal steroidogenesis in non-infected cirrhosis patients with sterile ascites and patients with spontaneous bacterial peritonitis and to evaluate the relation between such changes and circulating IL-6 levels.

Methods

Patients

The study period was January 2004 to June 2005. A total of 26 male patients with a diagnosis of cirrhosis were included from patients admitted over this period to the Department of Hepatogastroenterology and Tropical Medicine in the Theodor Bilharz Research Institute, Cairo, Egypt. There were 13 patients with sterile ascites and 13 with spontaneous bacterial peritonitis. Cirrhosis was diagnosed by liver biopsy or by clinical, laboratory and/or ultrasound findings. The severity of cirrhosis was assessed according to Pugh's modification of Child's classification. There were 9 Pugh–Child grade A patients, 9 grade B, and 8 grade C.

Spontaneous bacterial peritonitis was diagnosed when there was positive ascitic fluid culture and polymorphonuclear leuko-

cyte cell count ≥ 250 cells/μL. Sterile ascites was diagnosed when there negative ascitic fluid culture and polymorphonuclear leukocyte cell count < 250 cells/μL. Exclusion criteria were: patients with hepatocellular carcinoma and/or portal thrombosis; other neoplasm; signs or symptoms of systemic inflammatory response syndrome according to previously published criteria [*12*]; upper gastrointestinal bleeding; intake of antibiotics in the preceding 2 weeks, including selective intestinal decontamination with norfloxacin; under treatment with corticosteroids or pentoxifylline; and refusal to participate in the study. No patient was receiving nonsteroidal anti-inflammatory drugs, or any other drug known to influence HPA functions.

For comparison, 10 healthy age-matched control male subjects were recruited and their state of health was validated by clinical examination and routine laboratory investigations. Informed consent was taken from all participants.

Laboratory analysis

Venous blood was collected from all subjects (patients and controls) between 09.00 and 11.00 hours, in sterile, prechilled glass tubes containing ethylenediaminetetraacetic acid (EDTA) for ACTH assay, and in tubes with no additives for the other measurements (cortisol, DHEAS, IL-6 and CRP). Blood samples were transported in ice water, centrifuged at 4 °C, and separated plasma and sera were stored immediately in adequate aliquots at 70 °C until assayed.

Enzyme immunoassays were used for measurement of plasma ACTH, serum cortisol, serum DHEAS (Diagnostic Systems Laboratories, United States of America), and serum IL-6 (Diaclone Research, France). Serum CRP concentration was determined immunoturbidimetrically (Randox Laboratories, United Kingdom, catalogue number 2572). The lower detection limits of the assays were: 1.2 pg/mL for ACTH; 2.76 nmol/L for cortisol; 40.6 nmol/L for DHEAS; 2 pg/mL for IL-6; and 0.2 mg/dL for CRP.

Data analysis

Data were summarized as means and standard deviation (SD). Comparisons between the groups were done using 1-way analysis of variance. Pairwise comparisons were performed using Scheffe multiple comparison tests. The strength of the association between the measurements was calculated using Pearson's correlation coefficient. Significance was set at $P < 0.01$.

Results

All patients fulfilling the inclusion and exclusion criteria during the period of the study who agreed to participate were considered for entry into the study. The clinical and analytical characteristics of the patients in the 3 study groups (controls, sterile ascites and spontaneous bacterial peritonitis) are shown in Table 1. Most of the laboratory tests showed significantly differences in the 2 patient groups compared with the control group. Patients and controls were very closely matched in age to avoid bias.

The mean levels of interleukin-6, C-reactive protein, hormones and specific ratios are summarized in Table 2. In brief, serum levels of serum IL-6 showed a significant increase and DHEAS a significant decrease in cirrhosis patients with sterile ascites and spontaneous bacterial peritonitis compared with healthy subjects ($P < 0.01$). Serum CRP was significantly increased and cortisol/CRP ratio significantly decreased when compared with controls ($P < 0.01$). On the other hand, increased serum levels of cortisol and the cortisol/DHEAS molar

Table 1 **Clinical characteristics and laboratory data in non-infected cirrhosis patients with sterile ascites, patients with spontaneous bacterial peritonitis and a healthy control group**

Variable	Sterile ascites (*n* = 13)		Spontaneous bacterial peritonitis (*n* = 13)		Control (*n* = 10)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	54.1	11.9	57.4	13.3	62.1	10.4
Blood tests						
Platelets (10^3/mm^3)	103.5	27.5[b]	102.1	42.1[b]	180.6	31.0[a]
Haemoglobin (g/dL)	10.92	2.03[b]	11.06	2.27[b]	12.83	1.15[a]
Prothrombin time (s)	17.19	3.75[a]	21.45	11.10[a]	12.48	0.80[a]
Liver function tests						
ALT (U/L)	54.54	21.22[a]	61.00	39.31[a]	13.30	1.42[b]
AST (U/L)	73.54	31.03[a]	59.85	30.10[a]	11.90	1.73[b]
Bilirubin (mg/dL)	1.37	0.83[a]	4.06	2.06[b]	0.56	0.27[c]
Albumin (g/dL)	2.47	0.41[c]	2.13	0.30[b]	3.88	0.39[a]
Total protein (g/dL)	7.20	0.97	7.21	0.98	7.74	0.59
Alkaline phosphatase (U/L)	152.6	58.1[a]	179.2	57.3[a]	86.2	10.7[b]
Kidney function tests						
Sodium (mEq/L)	130.9	6.2[b]	126.2	8.9[b]	140.5	3.2[a]
Potassium (mEq/L)	4.46	0.48	4.49	1.14	4.12	0.50
Urea (mg/dL)	37.62	12.97	44.15	16.18	43.70	4.60
Creatinine (mg/dL)	1.10	0.29[a]	1.32	0.63[a]	0.77	0.22[b]

P ***< 0.01 was considered significant. For each significant test, group means sharing the same letter (a, b or c) are not significantly different from each other.***
n ***= number of subjects; SD = standard deviation.***
ALT = alanine aminotransferase; AST = aspartate aminotransferase.

ratio were found in both diseased groups of patients in comparison with healthy subjects *(P* < 0.01), whereas non-significant changes of plasma ACTH concentration were observed. The ratios of serum DHEAS and serum cortisol in relation to serum IL-6 were significantly decreased in both diseased groups in comparison with controls (*P* < 0.01).

Discussion

In the current study we recorded a significant increase in serum IL-6 levels in patients with sterile ascites and spontaneous bacterial peritonitis compared with healthy controls. This confirms the suggestion that enhanced cytokine levels are a consequence of liver dysfunction and inflammation, a finding that was observed by Lee et al. [*13*]. IL-6 is also a potent stimulator of the HPA axis and ACTH release [*14,15*]. Moreover, it can act synergistically with ACTH on the adrenal glands to release cortisol [*14*]. Serum cortisol, however, showed a significant increase in the 2 diseased groups of patients compared with healthy controls, suggesting that cortisol secretion is augmented by another stimulating factor. Such a factor may be IL-6, which has been shown to directly

Table 2 **Values of interleukin-6, C-reactive protein, hormone measurements and specific ratios in non-infected cirrhosis patients with sterile ascites, patients with spontaneous bacterial peritonitis and a healthy control group**

Variable	Sterile ascites (*n* = 13)		Spontaneous bacterial peritonitis (*n* = 13)		Control (*n* = 10)	
	Mean	SD	Mean	SD	Mean	SD
Serum IL-6 (pg/mL)	45.85	40.80[b]	165.6	110.0[a]	14.60	14.37[c]
Serum CRP (mg/dL)	12.32	6.94[b]	65.69	27.40[a]	1.75	1.45[c]
Serum DHEAS (nmol/L)	162.8	71.7[b]	435.5	513.8[b]	1252.0	783.3[a]
Plasma ACTH (pg/mL)	6.00	3.81	5.02	0.80	6.00	5.00
Serum cortisol (nmol/L)	12.89	4.56[a]	15.87	3.93[a]	6.07	2.43[b]
Cortisol (nmol/L)/CRP (mg/dL)	1.69	1.65[b]	0.28	0.12[c]	6.04	5.87[a]
Cortisol/DHEAS (molar ratio)	0.26	0.14[a]	0.50	0.45[a]	0.07	0.04[b]
DHEAS (nmol/L)/IL-6 (pg/mL)	5.90	5.95[b]	2.28	1.08[b]	129.55	107.86[a]
Cortisol (nmol/L)/IL-6 (pg/mL)	0.39	0.19[b]	0.14	0.08[b]	0.67	0.37[a]

P ***< 0.01 was considered significant. For each significant test, group means sharing the same letter (a, b or c) are not significantly different from each other.***
n ***= number of subjects; SD = standard deviation.***
IL-6 = interleukin-6; CRP = C-reactive protein; DHEAS = dehydroepiandrosterone sulfate; ACTH = adrenocorticotropic hormone.

stimulate cortisol release from primary human adrenocortical cell cultures [*16*]. This might also be supported by the non-significant change in the serum cortisol/IL-6 ratio in the 2 groups of liver cirrhosis patients as compared with healthy subjects.

Indeed, we also observed a significant difference between the 2 patient groups as regards serum CRP levels, which is consistent with the presence of the inflammatory response in cirrhosis patients. The raised cortisol secretion, despite non-significant changes in plasma ACTH levels, is therefore consistent with its role in survival as an adaptation to a prolonged systemic inflammatory response, and suggests that in this setting IL-6 plays a beneficial role. However, we also demonstrated a significant increase in serum CRP and a significant decrease in serum cortisol/CRP ratio in the spontaneous bacterial peritonitis group compared with the healthy subjects or cirrhosis patients with sterile ascites. This observation, and the lack of a significant change in plasma ACTH levels in our patients, could be attributed to an adaptation of the HPA axis to the long-standing activation by inflammatory stimuli [*17*].

IL-6 is thought to be the most important cytokine in several age-dependent diseases [*18–21*]. Thus, the increase in IL-6 production during the process of ageing might be related to diminished DHEAS secretion, which in turn may be a significant cofactor in inflammatory and age-related diseases [*22*]. Therefore, it it not surprising in this study to observe that, in association with the significant elevation of serum IL-6 levels, cirrhosis patients in both diseased groups showed a significant decrease of serum DHEAS values when compared with healthy subjects. Moreover, serum DHEAS concentrations in relation to those of IL-6 (DHEAS/IL-6 ratio) showed a significant

decrease in the 2 groups of liver cirrhosis patients compared with controls. Hence, as expected, our results revealed a significant increase in serum cortisol/DHEAS molar ratio in the 2 groups of cirrhosis patients, indicating a shift of adrenal steroidogenesis to cortisol production at the expense of androgen production. The mechanism whereby adrenal cortisol secretion is maintained, while adrenal DHEAS secretion falls, is unknown [*23*]. In chronic inflammatory diseases, however, the reason for the inadequately low hormone production seems to be located in the adrenal gland but not in the hypothalamus or the pituitary gland [*24*].

Therefore, in the light of the recent evidence that DHEA provides an immunostimulatory influence opposing the effect of glucocorticoids [*25*], the benefit for the host defence systems of a sustained hypercortisolism in the presence of low levels of DHEAS is questionable, as a prolonged imbalance between immunosuppressive and immunostimulatory hormones of adrenocortical origin may participate in the increased susceptibility to complications of infections [*26*].

To conclude, our results revealed that the severity of bacterial peritonitis in liver cirrhosis is associated with enhanced serum IL-6 and cortisol levels, and a decrease in serum DHEAS, in relation to serum IL-6 concentrations. Careful, prudent and relatively long-term studies on DHEA administered to cirrhosis patients may progressively assess its safety and its ability to prevent and/or improve a number of pathological conditions that complicate liver cirrhosis. Research that focuses on these issues should provide further important insights into the links between the immune system and the HPA, and will provide further evidence that these interactions have important physiological roles in health and disease.

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