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Serial Changes in Plasma Annexin A1 and Cortisol Levels in Sepsis Patients

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Abstract

Annexin A1 (AnxA1), originally identified as a glucocorticoid-regulated protein, is an important endogenous anti-inflammatory mediator during the resolution phase of inflammation, and its circulating level has been rarely studied in sepsis patients. Glucocorticoid has been extensively used in treating patients with sepsis. However, it is unclear whether endogenous cortisol or exogenous glucocorticoid contributes to the regulation of AnxA1 levels in peripheral blood of sepsis patients. The aim of this study was to investigate: [1] serial changes over time in the plasma levels of AnxA1 and cortisol in sepsis patients; and [2] prognostic value of AnxA1 level in the survival of sepsis patients. Fifty-eight adult sepsis patients admitted to an intensive care unit (ICU) were enrolled. The plasma levels of cortisol and AnxA1 were determined by specific enzyme-link immunosorbent assay. Results show that the median daily levels of cortisol at the 1st, 3rd, 5th and 7th day after admission to ICU were significantly elevated over the cortisol level of the control subjects. However, the AnxA1 level was elevated in only thirty-three patients (56%) over the observation period. There was no significant correlation between cortisol levels and AnxA1 levels. Further analysis indicated that steroid treatment resulted in significant elevation of the cortisol level over time, but did not affect the AnxA1 level. AnxA1 levels were also not statistically different between surviving and non-surviving patients. In conclusions, the circulating level of AnxA1 is elevated in a subgroup of sepsis patients, and the AnxA1 level does not correlate with the cortisol level in the peripheral blood of sepsis patients.

Key Words: acute lung injury, annexin A1, anti-inflammatory mediators, cortisol, sepsis

Introduction

The inflammatory response is a protective process whereby the body is able to counteract infections or other localized insults (16). Polymorphonuclear leukocytes, monocytes/macrophages, lymphocytes and endothelial cells are the cellular effectors of the inflammatory response. Activation of these cells at the site of the systemic or local insult leads to a release of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6), which are able to induce transmigration of leukocytes into the inflammatory areas (1, 9). If excessively activated, these cells produce oxygen-free radicals and other cytotoxic products, which may cause organ failure or even death (16, 27). In order to restore normal homeostasis, the pro-inflammatory phase is able to simultaneously trigger various endogenous anti-inflammatory mechanisms that carry out the timely introduction and removal of various leukocyte subsets; this finally lead to the resolution phase of inflammation (20, 30, 32, 33). Previous studies have reported that the levels of anti-inflammatory cytokines, such as IL-10, IL-6, soluble tumor

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necrosis factor I (sTNF RI), sTNF RII and IL-1 receptor antagonist (IL-1Ra), are also significantly elevated in patients with sepsis (9, 12, 19). However, there is still no well-accepted biomarker that can be used to monitor anti-inflammatory activities in a clinical setting.

Annexin A1 (AnxA1) was originally identified as a glucocorticoid-regulated protein and is an endogenous anti-inflammatory mediator during the resolution phase of inflammation. It is particularly abundant in various cells of the host immune system, including monocytes, macrophages, T lymphocytes and neutrophils (4, 5, 24, 25). AnxA1 can be mobilized from cytoplasm to membrane and is eventually released in abundance from neutrophils after activation or after adherence to endothelial cells (4, 24, 25). Functionally, AnxA1 has been shown to attenuate leukocyte recruitment in many experimental inflammatory models by inhibiting cell adhesion and transmigration (22, 24). The important anti-inflammatory role of AnxA1 in the normal defense mechanisms of the body has been further supported by animal studies showing that AnxA1-null mice are more prone to both acute and chronic inflammatory reactions (6, 8, 15, 17, 18, 26, 36, 39). These changes occur because neutrophils defective in AnxA1 exhibit higher levels of activation and chemotaxis in response to various stimuli. In the clinical setting, AnxA1 has been implicated in various human pulmonary disorders including cystic fibrosis and the acute exacerbation of idiopathic pulmonary fibrosis (8, 17, 18, 36). However, the level of AnxA1 in the peripheral blood of patients with sepsis or inflammatory diseases has been rarely determined, and little is known about its clinical implications.

Endogenous glucocorticoids play an important role in regulating the host-defense system. Cortisol levels are elevated under conditions of stress such as critical illness and severe sepsis/septic shock (3, 23, 28, 29, 38). Dysregulation of the secretion and/or activity of endogenous cortisol would seem to compromise immune/inflammatory cell function and thereby disrupt homeostatic physiology (23). Glucocorticoids upregulate AnxA1 protein production in both granulocytes and macrophages, and have also been found to enhance the release of AnxA1 by macrophages (3). Glucocorticoids have been extensively used with variable success for the treatment of patients with sepsis or acute lung injury (16, 21, 27). However, the clinical roles of endogenous cortisol and exogenous glucocorticoids with respect to the circulating levels of AnxA1remain unclear. On this basis, we conducted a study to determine firstly the levels of AnxA1 and cortisol in the peripheral blood of sepsis patients over a time span, and secondly the prognostic value of AnxA1 level in the survival of sepsis patients.

Materials and Methods

Patients

This was a prospective, observational study performed at a tertiary referral intensive care unit (ICU). The study was approved by the Ethics Committee of the Taipei Medical University Hospital, and informed consents were signed by either the patients themselves or their next of kin. Patients were recruited on a consecutive basis over the period June 2008 to May 2010. Patients with a diagnosis of septic shock were screened for eligibility. The inclusion criteria consisted of a clinical suspicion of infection and evidence of a systemic response to infection; these needed to fulfil at least two of four criteria related to the presence of systemic inflammatory response syndrome and to evidence of shock. The criteria for systemic inflammatory response syndrome and shock were as published previously (2).

Measurements of Cortisol and AnxA1

Peripheral blood samples were collected from patients during the first day after admission to the ICU and thereafter every other day until day 7. Peripheral blood samples from 20 healthy adult persons were also collected for use as normal controls. Peripheral blood was centrifuged at 250 $\times g$ for 10 min and each plasma sample was stored at -80°C until the levels of mediators were measured. The levels of cortisol were determined by a commercial kit (R&D Systems, Minneapolis, MN, USA), and the level of AnxA1 was determined as reported by Goulding *et al*. (14).

Statistical Methods

All statistical tests were performed using SPSS software for Windows (SPSS, Chicago, IL, USA). The Mann-Whitney nonparametric test was used to compare continuous outcome measures between patients and controls, and between survivors and non-survivors. P value < 0.05 was accepted as significant difference. Changes in cortisol and AnxA1 concentrations over time were assessed in order to determine the presence of a linear trend using the mixed model approach. Spearman rank correlation was used for estimating the correlation between cortisol levels and AnxA1 levels.

Results

Characteristics of the Patients

A total of 58 patients were recruited into this

Table 1. Characteristics of the patients

Characteristics	Total	Outcome			Treatment		
		Survival	Non-survival	*P*-value	Steroid	No steroid	*P*-value
Number	58	37	21		26	31	
Age	75.0 ± 12.9 (44-95)	75.5 ± 13.5 (44-93)	74.2 ± 12.1 (50-95)	0.286	73.3 ± 12.7 (44-86)	76.4 ± 13.1 (48-95)	0.956
BMI	22.0 ± 5.8 (14.7-52.2)	22.0 ± 6.7 (14.7-52.2)	21.9 ± 3.9 (15-31.3)	0.296	20.1 ± 2.6 (15-24)	23.3 ± 7 (14.7-52.2)	0.096
APACHE II	22.0 ± 8.1 (6-37)	21.8 ± 6.6 (12-37)	17.9 ± 9.4 (6-35)	< 0.05	18.0 ± 7.9 (6-32)	21.8 ± 8.0 (8-37)	0.980
Ventilator Tx	68.4%	70%	63.6%	0.665	71.9%	65.9%	0.66
Steroid Tx	42.1%	38.3%	53.2%	< 0.05	100%	0%	
Hospital days	36.3 ± 30.7 (4-163)	40.9 ± 33.7 (10-163)	28.3 ± 23.2 (4-100)	0.349	39.9 ± 29.7 (4-105)	33.2 ± 31.7 (9-163)	0.712
White blood cell count	11,735 ± 6,161.3 (750-34450)	11,692 ± 5,662 (4590-29700)	11,859 ± 7,467 (750-34450)	0.377	10,790 ± 5,758 (750-28030)	11,965 ± 6,183 (4910-34450)	0.69
Absolute neutrophil count	83.1 ± 11.0 (37.3-99.1)	83.2 ± 10.8 (55.1-99.1)	82.8 ± 11.7 (37.3-97.9)	0.449	82.1 ± 12.2 (37.3-97.9)	83.1 ± 10.0 (57.8-99.1)	0.24
CRP (mg/dl)	10.0 ± 7.7 (0.28-27.5)	9.7 ± 7.3 (0.78-27.5)	10.3 ± 8.5 (0.28-22.5)	0.679	9.3 ± 7.3 (0.28-21.73)	10.7 ± 8.2 (0.78-27.5)	0.468
Cortisol (ng/ml)	452 ± 145 (17-12599)	244 ± 176 (17-1221)	891 ± 1,994 (268-12599)	< 0.001	704 ± 1726 (17-12599)	268 ± 188 (43-1165)	< 0.001
Annexin A1 (ng/ml)	25.3 ± 60.2 (0.1-453.4)	27.2 ± 63.8 (0.1-453.4)	16.3 ± 38.6 (0.1-194.9)	0.222	33.0 ± 77.3 (0.2-453.4)	14.5 ± 18.9 (0.1-95.5)	< 0.05

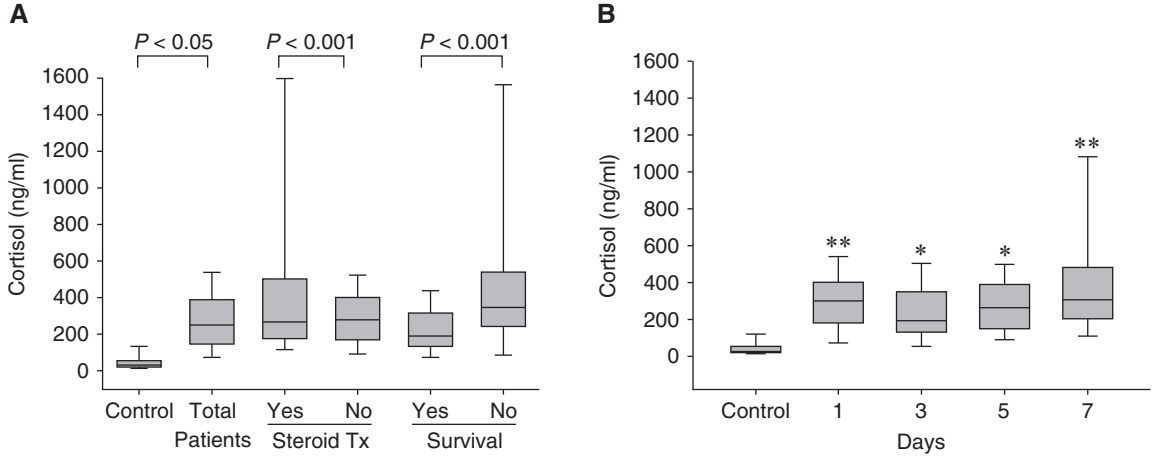

Fig. 1. (A) Changes in the cortisol level in sepsis patients and control individuals. (B) The changes over time in the cortisol levels of all sepsis patients during the 7-day study period. Boxes present the interquartile range bounded by the 25th and 75th percentile with the horizontal bar as the median. The whiskers represent the distribution of values from the 5th to 95th percentile. *P*-values were as compared with normal control levels: **P* < 0.05, ***P* < 0.001.

study, including 44 male (75%) patients. The characteristics of these patients are shown in Table 1. The source of sepsis was classified as pulmonary in 47 cases (82%), gastrointestinal in 4 cases (6%), neurological in 5 cases (9%), and urosepsis in 2 cases (3%). Of the recruited patients, 24 individuals (42%) received steroid treatment during the study period. No significant difference in the clinical parameters between patients with or without steroid treatment was found. In total, 21 patients (36%) died within 28 days of admission to the ICU. Further analysis showed that the non-surviving patients were associated with more frequent usage of steroid treatment than the surviving patients (53.2% *vs*. 38.3%; $P < 0.05$).

Serial Changes in the Level of Cortisol Over Time

The median total cortisol levels in plasma over the complete study period is shown in Fig. 1A. It can be clearly seen that there was a significant el-

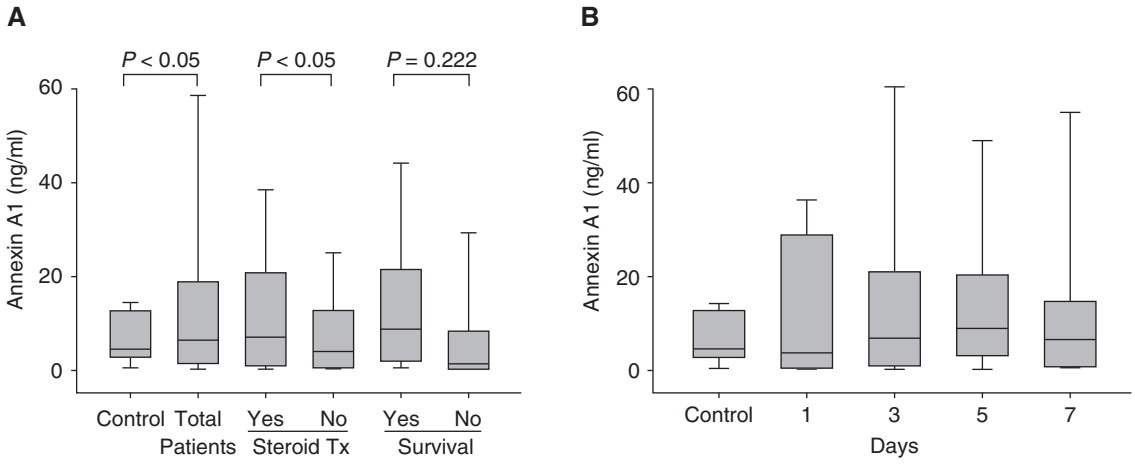

Fig. 2. (A) Changes in the AnxA1 level in sepsis patients and control individuals. (B) The changes over time in the AnxA1 levels of all sepsis patients during the 7-day study period. Boxes present the interquartile range bounded by the 25th and 75th percentile with the horizontal bar as the median. The whiskers represent the distribution of values from the 5th to 95th percentile.

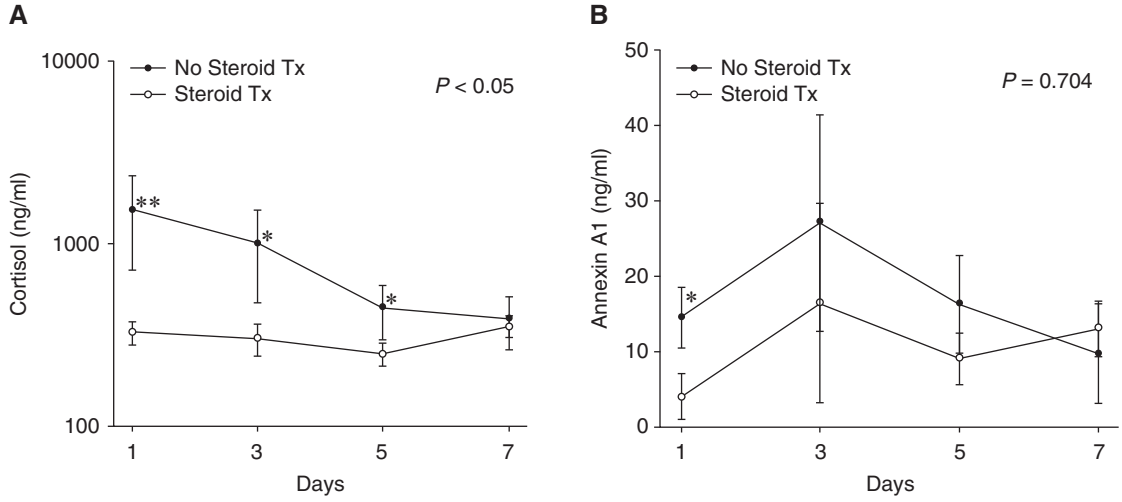

Fig. 3. Comparison over time of cortisol (A) and AnxA1(B) levels during the 7-day study period between patients with and without steroid treatment. *P*-values were as compared with normal control levels: $*P < 0.05$, $**P < 0.005$.

evation of cortisol level in sepsis patients compared to the control subjects ($P < 0.05$). All patients had an elevated level of cortisol over the normal control level during the 7 day observation period. The median daily levels of cortisol at the 1st, 3rd, 5th and 7th day after admission to ICU were also significantly elevated over those of the control subjects ($P < 0.001$, $P < 0.05$, $P < 0.05$ and $P < 0.001$, respectively; Fig. 1B). There was no significant change over time in cortisol levels of the patients during the study period.

Serial Changes in the Level of AnxA1 Over Time

The AnxA1 level was elevated in only thirty-three patients (56%) over the 7-day observation period (Fig. 2B). Fig. 2A shows that the median level of total AnxA1 as determined for the complete study period was significantly elevated over those of the control subjects ($P < 0.05$); however, the daily levels of the patients over time were not significantly different from the levels of the control individuals (Fig. 2B).

Effect of Steroid Treatment on the Levels of Cortisol and AnxA1

The median levels of total cortisol and AnxA1 in patients with steroid treatment were both significantly higher than the corresponding levels in patients without steroid treatment ($P < 0.001$ and $P < 0.05$, respectively) (Figs. 1A and 2A). The effects of steroid treatment on the changes over time of both mediators during the study period were next determined. Steroid treatment was able to significantly enhance the levels of cortisol over time ($P < 0.05$) and

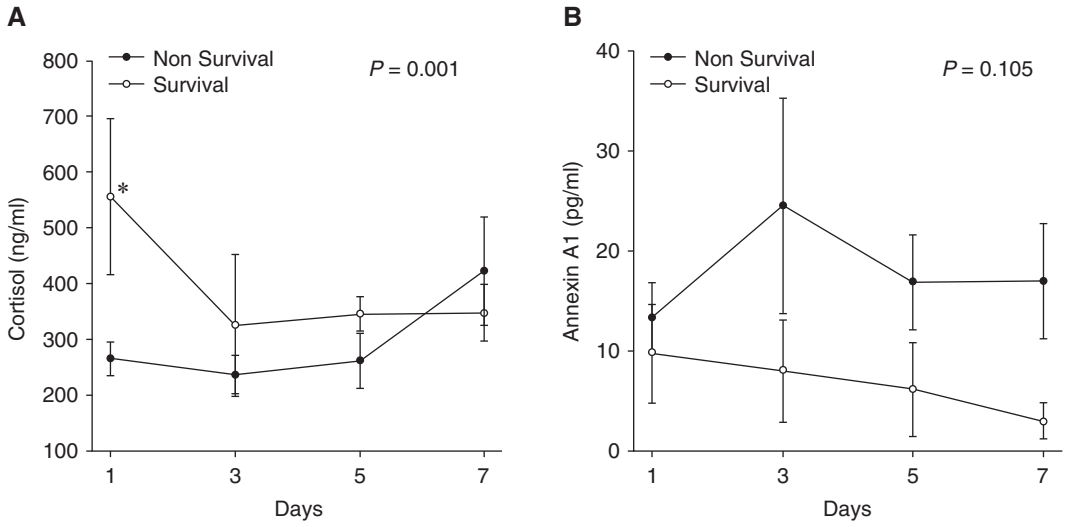

Fig. 4. Comparison over time of cortisol (A) and AnxA1 (B) levels during the 7-day study period between surviving and non-surviving patients. *$P < 0.005$.

that this effect was especially profound on day 1, day 3 and day 5 ($P < 0.005$, $P < 0.05$ and $P < 0.05$, respectively) (Fig. 3). However, steroid treatment did not significantly elevate the levels of AnxA1 over time ($P = 0.704$; Fig. 3B), although there was a significant increase in the AnxA1 level on day 1 ($P < 0.05$). It was also observed that there were 17 patients whose serial AnxA1 levels were not elevated at all and, among these subjects, 10 patients (58%) had undertaken steroid treatment.

Correlative Analysis

A correlative analysis was carried out and there was no significant correlation between the levels of cortisol and AnxA1 ($R = -0.046$, $P = 0.735$). This lack of correlation remained even when the patients receiving steroid treatment ($R = 0.048$, $P = 0.772$) were split from those not receiving steroid treatment ($R = -0.014$, $P = 0.947$).

Relationship between Survival Outcome and the Levels of AnxA1 or Cortisol

We compared the total levels of both mediators between the surviving and non-surviving patients. The median level of total cortisol was significantly higher among the non-surviving patients ($P < 0.001$; Fig. 1A) than among the surviving patients, and the changes in level over time in the non-surviving patients were also significantly larger than the corresponding changes in the surviving patients ($P = 0.001$; Fig. 4A). However, there was no significant difference in either the median total AnxA1 level ($P = 0.222$; Fig. 2A) or daily AnxA1 levels over time ($P = 0.105$; Fig. 4B) between the non-surviving and surviving patients.

Discussion

This study examined changes in AnxA1 and cortisol levels in sepsis patients over a 7-day period. The results demonstrated that in response to infectious insults the level of AnxA1 was elevated in the peripheral blood of only fifty-six percent of sepsis patients over the observation period. In consistent with previous studies (23, 28, 29), the level of cortisol was significantly elevated in all patients with sepsis; however, these levels did not closely correlate with the level of AnxA1. Moreover, steroid treatment did not result in the elevation of the serial levels of AnxA1 over time in the sepsis patients. A previous study has reported that AnxA1 lacks a signal peptide and cannot be exported *via* any classical secretary pathway (11). We have also reported that exogenous glucocorticoid does not affect the production of AnxA1 protein in leukocytes nor does it affect the release of AnxA1 by leukocytes (34). Nevertheless, glucocorticoid is able to reduce the expression of AnxA1 in T-lymphocytes and thereby inhibits T-lymphocyte activation (5, 6). Therefore, the discrepancy between serum levels of glucocorticoid and annexin A1 could be related to these effects.

Our results demonstrate that the circulating level of AnxA1 was elevated in only a subgroup of sepsis patients. The elevation of AnxA1 level in these patients is likely due to the release of AnxA1, in either free form or microparticles, by activated neutrophils or monocytes/macrophages in response to the infectious insults (7, 24-26, 35). In addition, AnxA1 is

also released by apoptotic neutrophils during the process of inflammation (26, 31, 32). AnxA1 both inhibits leukocyte recruitment into inflamed tissues and enhances the clearance of apoptotic cells by tissue macrophages (16, 27); furthermore, the AnxA1-containing microparticles are also able to mediate a rapid anti-inflammatory effect with respect to cell-to-cell interactions (7, 35). Taken together, our results imply that an elevated level of AnxA1 in the peripheral blood of sepsis patients may play an active anti-inflammatory role which subsequently contributes to the resolution of sepsis. In parallel with this supposition, a previous study also indicated that a greater anti-inflammatory response resulted in a less severe sepsis (37).

It is still a controversial issue as to whether or not the circulating level of anti-inflammatory mediators can be used as a prognostic factor with respect to patient survival. Previous studies have reported that an early response to continuously elevated anti-inflammatory cytokine levels, such as IL-10, sTNF-RI, sTNF-RII and sIL-1Ra, is associated with an enhanced risk of a fatal outcome for sepsis patients (9, 10, 13, 14, 31). However, our results demonstrate that there were forty-four percent of patients whose AnxA1 levels were not elevated during the observation period. The interpretation of our findings was limited by the relatively small number of patients involved in this study. It is unclear why the circulating AnxA1 levels of the subgroup of sepsis patients were not elevated in response to septic insults. This warrants further studies to investigate the role of circulating AnxA1 level in clinical applications among patients with sepsis.

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