Loss of serum transcortin in human shock associated with severe infection by candida albicans

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Abstract. The cortisol binding ability of transcortin (corticosteroid binding globulin or CBG) was found to be virtually absent from the sera of patients in a state of shock associated with high levels of antibodies to candida albicans.

At the same time the serum proteins of the patients in shock displayed a number of the classical responses seen in acute inflammation: increased haptoglobin (28 ± 10 vs 10.8 ± 7.7 μM in normals), reduced prealbumin (1.7 ± 0.6 vs 3.6 ± 0.1 μM in normals) and albumin (485 ± 78 vs 600 ± 200 μM in normals).

The shock sera also showed increases in levels of endogenous cortisol (610 ± 260 vs 395 ± 192 nm in normals) and progesterone (2.40 ± 0.7 vs 1.4 ± 1.2 nm in normals) similar to those seen in inflammatory conditions.

In sera from patients with superficial chronic candidiasis no significant variation of cortisol binding was observed. Haptoglobin was increased (26.4 ± 8.2 μM) and prealbumin decreased (2 ± 0.8 μM), while the other serum indices tested retained essentially normal levels.

Compared with previous studies from this laboratory which demonstrate a fall of CBG in septic shock of bacterial origin and lack of this response in acute inflammation, these results suggest that the loss of serum CBG activity is a specific marker for shock of fungal or bacterial aetiology.

Possible endocrine or immune implications and clinical applications of our findings are discussed.

Low transcortin (CBG) values have been described in a few cases of liver, adrenal or renal disease (Brien 1981) or as a result of surgery (Hamanaka et al. 1970). Until recently, however, no consistent decrease of this corticosteroid binding activity has been reported in human disease states.

Studies from this laboratory have shown that a severe and highly reproducible fall of CBG activity occurs in the rat as a result of turpentine-induced inflammation (Savu et al. 1980) and in man during the early phase of septic shock of bacterial aetiology (Savu et al. 1981). In man, this response resulted in virtual disappearance of the glucocorticoid binding activity of the serum; this seemed specific for septic shock, since sera from patients with shock or acute inflammation displayed similar responses of their classical acute phase reactants (APRs), and endogenous cortisol or progesterone levels, whereas the CBG fall was only seen in the shock sera.

We have now extended our observations to shock associated with severe acute infection by the fungus candida albicans and shown that this disorder brings about an almost complete loss of CBG activities similar to that caused by bacterial septic shock.

In addition, to further delineate the specificity of the CBG fall in various shock and inflammatory conditions, we have examined the serum levels of typical APRs, steroids, and thyroid hormones, in patients with severe or superficial candidiasis.

The physiological and practical implications of our findings are discussed.

Material and Methods

Sera

Three groups were studied: sera from patients in shock associated with severe acute infection by candida alb-
cans, sera from patients with superficial chronic candidiasis and control sera.

The shock sera originated from 22 adult in-patients of both sexes (11 males; 11 females). Their clinical course was as follows: admission to hospital for acute infections of various aetiology; treatment with antibiotics; persistence of high fever after 8–9 days of antibiotic treatment; at this time, clinical diagnosis of shock based at least on cold and mottled skin and urine flow under 30 ml/h; and serological diagnosis of severe candida albicans infection based on: a) passive haemagglutination tests (PHA) with sensitised sheep erythrocytes from Roche Laboratory, Paris, France (Zouaghi & Gauthier 1980), with titres of at least 1/160 (mean titre value for the 22 studied sera 1/558 ± 291); b) positive electrosyneresis.

One patient (female, 45 years old, respiratory insufficiency) was followed over a 38 day period, from the day of admission up to recovery.

The superficial candidiasis sera originated from 13 adult out-patients of both sexes (5 males, 8 females) with chronic oral, vaginal, urinary or digestive mucocutaneous candidiasis, detected by microbiological methods; PHA titers were less than 1/80 and electrosyneresis was negative.

The control sera were collected from 15 healthy male and female donors receiving no medication; PHA titers were less than 1/80.

Steroids:
The following radioactive steroids of 97–98% regularly checked purity were used: [1,2,6,7-3H]cortisol, 52 Ci/mmol; [1α, 2α,(n)-3H]progesterone, 55 Ci/mmol (Radio-chemical Centre, Amersham).

Binding studies
The binding of cortisol and progesterone by the sera was measured at equilibrium, using a batchwise dialysis technique, with a suspension of Sephadex G25 as the semi-permeable membrane (Pearlman & Crépy 1967). The binding activities were expressed as '1/P' binding indices, (1/g), where P is the concentration of serum proteins corresponding to an equilibrium ratio of steroid unbound/steroid bound = 1. Detailed descriptions of the method and the calculations have been given elsewhere (Savu et al. 1977).

Total protein concentrations of the sera were measured according to Lowry et al. (1951).

Stripping of endogenous steroids was performed by the addition of charcoal (Westphal 1971a); stripped and native homologous sera yielded identical 1/P binding indices.

Haptoglobin, prealbumin, albumin and thyroxine binding globulin (TBG) determination
The levels of the individual plasma proteins were evaluated by radial immunodiffusion, using Partigen kits from BEHRING (Paris, France).

Endogenous hormone determinations
Cortisol and progesterone concentrations were analysed by conventional radioimmunoassay, with specific rabbit antisera from the Institut Pasteur, Paris; thyroxine (T4) and triiodothyronine (T3) were evaluated by enzyme immunoassay (Zouaghi 1981) using ELISA systems (BOEHRINGER, Mannheim, Germany).

Table 1.
Response of 1/P serum binding indices for cortisol and progesterone in severe (with shock state) and superficial candida albicans infections.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± se; 1/g</th>
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<tbody>
<tr>
<td></td>
<td>Cortisol</td>
</tr>
<tr>
<td>Shock</td>
<td>0.6 ± 0.4a</td>
</tr>
<tr>
<td>Superficial</td>
<td>3.3 ± 0.5b</td>
</tr>
<tr>
<td>Normal</td>
<td>2.7 ± 0.9</td>
</tr>
</tbody>
</table>

a Statistically significant compared with normal (P < 0.001 by Student's t-test).

b Non-significant.
Table 2.
Response of haptoglobin, prealbumin, thyroxine binding globulin (TBG) and albumin in severe (with shock state) and superficial candida albicans infections.

<table>
<thead>
<tr>
<th></th>
<th>No. of subjects</th>
<th>Mean ± se; µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Haptoglobin</td>
</tr>
<tr>
<td>Shock</td>
<td>22</td>
<td>28.3 ± 9.1a</td>
</tr>
<tr>
<td>Superficial</td>
<td>13</td>
<td>26.4 ± 8.2a</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>10.8 ± 7.7</td>
</tr>
</tbody>
</table>

\(^a\) Statistically significant compared with normal (\(P < 0.01\) by Student’s t-test).

\(^b\) Statistically significant compared with normal (\(P < 0.05\)).

\(^c\) Non-significant.

Results

Binding of cortisol and progesterone in sera from patients with severe or superficial candida albicans infection

Table 1 presents the 1/P binding indices measured at equilibrium for cortisol and progesterone in sera from patients undergoing shock associated with high positive anti-candida immune response (PHA titres from 1/160 to 1/5120). These are compared to patients with chronic candidiasis and anti-candida PHA responses less than 1/80 and to normals with PHA responses also less than 1/80.

In the shock cases, the binding of the two steroid hormones is dramatically decreased. The interaction with cortisol, which is essentially due to CBG (King & Mainwaring 1974; Ballard 1979), has virtually disappeared, while the binding of progesterone, which involves the contribution of both CBG and \(\alpha_1\)-acid glycoprotein (Westphal 1971b) is affected to a lesser degree. In the superficial candidiasis patients, no such response is observed. The binding of the steroids in this category even appears slightly, though not significantly, more elevated than in the healthy controls.

Haptoglobin (Hp), thyroxine binding prealbumin (TBPA), thyroxine binding globulin (TBG) and albumin levels in sera from patients with severe or superficial candidiasis

The concentrations of several plasma proteins, functioning as APRs and/or hormone binders, were measured in the pathological and normal sera under study. The results are presented in Table 2. It may be seen that the shock sera display variations of Hp, TBPA and albumin similar to those characteristic for acute inflammatory diseases (Gordon 1976), i.e. strong Hp increments and marked decreases of prealbumin and albumin.

TBG has recently been reported to increase slightly during certain inflammations (Zouaghi 1981), but levels remained in the normal physiological range during shock.

The cases of superficial candidiasis had some inflammatory features, since statistically significant Hp increases and prealbumin decreases were observed.

Endogenous cortisol, progesterone, thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) levels in sera from patients with severe or superficial candidiasis

We have previously demonstrated in sera from patients with bacterial septic shock or acute inflammation important rises of cortisol and progesterone, two privileged endogenous ligands of human CBG (Savu et al. 1981). We show in Table 3 similar increases of cortisol and progesterone in the candida shock sera, in contrast to unchanged levels in superficial candidiasis.

Table 3 gives data concerning the thyroid hormones. Levels of T\(_4\) in shock or superficial candida patients do not differ from normals, whereas T\(_3\) tends to decrease in the shock sera; an analogous trend of T\(_3\) decrease was recently reported in a number of inflammatory cases (Zouaghi 1981).

It may be concluded that the responses of protein and hormone plasma parameters are broadly
Table 3.
Response of cortisol, progesterone, thyroxine (T₄) and triiodothyronine (T₃) levels in severe (with shock state) and superficial candida albicans infections.

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Mean ± SE; nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortisol</td>
</tr>
<tr>
<td>Shock</td>
<td>610 ± 260ᵃ</td>
</tr>
<tr>
<td>Superficial</td>
<td>380 ± 170ᵇ</td>
</tr>
<tr>
<td>Normal</td>
<td>395 ± 192</td>
</tr>
</tbody>
</table>

ᵃ Statistically significant compared with normal (P < 0.05 by Student's t-test).
ᵇ Non-significant.

similar in candida shock, bacterial shock, acute inflammations and, to a lesser extent, in superficial candidiasis. In contrast, the loss of CBG emerges as a specific response of the state of shock, whether of bacterial or fungal aetiology.

Kinetic studies in one patient: cortisol binding, anti-candida antibodies, haptoglobin and endogenous cortisol

In one patient we had the opportunity to follow the various serum parameters from the day of admission with respiratory insufficiency and high fever.

Fig. 1.
Kinetics of serum cortisol binding indices 1/P, anti-candida passive haemagglutination titers and cortisol concentrations, in a case involving a period of shock associated with candida albicans infection.
(day 0) though the periods of antibiotic treatment (days 0—9), shock and severe candidiasis (days 10—14), and gradual recovery (up to day 38). The more significant results are illustrated in Fig. 1, which compares the kinetics of cortisol binding, anti-candida antibodies, and endogenous cortisol concentrations. The course of the CBG decrease and subsequent recovery strikingly paralleled the rise and subsequent normalization of the antifungal response. On the other hand, the CBG peak was coincident with a positive peak of cortisol. Haptoglobin, a classical APR, (results not shown), retained high inflammatory levels throughout the disease (days 0—26), with no specific variations during the shock interval.

Discussion

The present study reveals that sera from patients in a state of shock associated with high levels of anti-candida antibodies are virtually depleted of the cortisol binding ability characteristic of transcortin. Besides, these sera display a number of inflammatory features, i.e. increases of haptoglobin, cortisol and progesterone, as well as reductions of thyroxine binding prealbumin and albumin.

Loss of CBG activity with inflammatory reactions similar to those described in this study were reported in patients in the early phase of septic shock of bacterial origin (Savu et al. 1981). In contrast, we did not find any significant CBG variation either in human acute inflammation or, currently, in superficial chronic candidiasis. Also, to the best of our knowledge, there are no reports of diseases with disappearance of CBG activity similar to that demonstrated for the shock cases of fungal or bacterial aetiology. The sum of available data thus points to the loss of CBG activity as a consistent and highly specific response of the shock condition.

We have previously presented evidence, mainly based on inhibition experiments, that the fall in glucocorticoid binding activities is unlikely to arise from interference of readily exchangeable endogenous inhibitors (Savu et al. 1981). This is confirmed by the observation that the removal of the endogenous corticosteroids from shock sera by the classical charcoal technique does not restore normal CBG levels. The loss of CBG activity might therefore be attributed either to the actual leakage of transcortin from the serum or to the occurrence during shock of strongly bound inhibitors that are not eliminated by the conventional ‘stripping’ techniques. Both phenomena could be involved. To clarify these points we are currently elaborating the immunological assay of human CBG and analysing various extracts from pathological and normal sera for the presence of possible CBG inhibitors.

While the mechanisms underlying the deficiency of corticosteroid binding ability remain to be clarified, an evaluation of its physiological implications may already be attempted.

It should be noted that all proteins which decrease in shock due to candida albicans are hormone binders, whether they interact weakly and non-specifically, like albumin, or with high affinity and specificity, as do the transcortin-cortisol and prealbumin-T4 systems. The consequence must be a relative rise of free hormones, and hence, as now commonly accepted (Nunez et al. 1979), a potentiation of their effects. The response of the CBG-cortisol couple would be the most effective, owing to the highly divergent associated changes of the protein binder and of its steroid ligand. The resulting enhanced impact of the steroid may be involved in defensive anti-inflammatory and immune mechanisms during candida albicans shock.

The bearing of our results on immunological problems is of special interest. In all the patients with shock, the same correlations were observed of increased anti-candida antibodies, increased cortisol and loss of CBG. The indications given by the kinetic survey of one patient, though admittedly preliminary, re-inforced the notion of sustained temporal correlations between the CBG decrease and the rise of specific antibodies. It is now well documented that changes in hormone concentrations can affect immune performance considerably (Maor et al. 1974; Fauci 1979). Conversely, immune responses may themselves trigger hormonal changes, in particular large increases in glucocorticoids (Besedovsky et al. 1975). The simultaneously elevated levels of cortisol and antibodies in the candidiasis shock are highly suggestive of mutual endocrine-immune influences, and the concomitant loss of CBG activity might further facilitate interactions of this kind. It is even tempting to speculate that the loss of CBG in itself, i.e. independently of its binding capacity, could be a stimulating factor of the immune response, since an inhibitory role of CBG in immunity has been reported (Amaral & Werthamer 1976). As yet, however, cause and effect relationships cannot be
extrapolated from the temporal correlations observed between hormone, binding protein and antibody levels.

Another important implication of the simultaneously decrease in CBG activity and increased corticosteroid levels concerns the control mechanisms of adrenal hormone secretion. The greatly enhanced concentration of unbound glucocorticoids is liable to exert a feedback inhibitory action at the level of the hypothalamo-hypophysal axis, resulting in reduced ACTH secretion and subsequent inhibition of glucocorticoid production by the adrenal cortex. The gradual restoration of the normal cortisol levels in the case when recovery from shock occurs, probably involves steroid-brain interactions of this kind, and the loss (or masking) of CBG activities might significantly contribute to this metabolic normalization.

In the light of our results, assay of the serum corticosteroid binding ability appears as an advisable, easily performed test, to be included in the clinical investigations aimed at establishing a biochemical diagnosis of shock caused by bacterial or fungal infections. The importance of a sensitive, reproducible and specific test for the shock associated with severe candidiasis is stressed by the increasing occurrence of this disorder, often subsequent to intensive antibiotic or corticosteroid treatments (Torack 1957; Vachon & Veysier 1975).

We are currently accumulating kinetic evidence for the prognostic value of the CBG response in the development of infectious shock. Our investigations are also being extended to cases of traumatic non-infective shock.

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