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Blood Coagulation Changes in Response to Dexamethasone in the Normal Rat

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Abstract

The effect of daily dexamethasone administration on blood coagulation was studied in normal male rats. Dexamethasone was injected daily (0.4 mg/kg body weight) for 3 weeks. Blood was collected retro-orbitally from the rats before, after 2 days, after 1 week and after 3 weeks of dexamethasone administration. Prothrombin time (PT), fibrinogen (FIB) and activated partial thromboplastin time (APTT) were determined in plasma. The results were compared to the values found in normal adult male rats before injections (controls). It was found that dexamethasone significantly increased the FIB already after 2 days of administration, while it significantly decreased APTT starting after 1 week of dexamethasone injections. PT, however, remained unaffected by dexamethasone throughout the time of the study. From these results it can be concluded that dexamethasone does not seem to affect the extrinsic coagulation cascade, but increases procoagulant activity via the intrinsic pathway. Also decreased fibrinolysis was recorded, as witnessed by increased fibrinogen levels. Thus, glucocorticoids should be prescribed with caution in patients with hypercoagulable states.

Introduction

GLUCOCORTICOIDS are commonly used in the therapy of disorders with an increased clotting tendency, such as immune complex diseases, nephrosis and malignant hematologic disorders. So far, the effect of glucocorticoids themselves on the blood coagulation system is not resolved Some investigators reported a state of hypercoagulability in association with glucocorticoids [1,3], while others demonstrated the opposite effect [4,5]. More recent studies have examined the effect of glucocorticoids on blood coagulation in various disease states, so that the effect of glucocorticoids per se could not be clearly determined [6,7]. The aim of this study was, therefore, to compare the results of coagulation tests before, after short and more prolonged administration of glucocorticoids in healthy adult male rats to avoid the interference of an underlying disease condition.

Material and Methods

Experimental Animals:

Six adult male albino rats weighing 150-200 gram were used in this study. They were allowed free mobility and access to food and drinking water. A regular therapeutic dose of dexamethasone (0.4

mg/kg body weight) was injected intraperitoneally [6] daily for 3 weeks. Blood samples were taken retro- orbitally at four time incidences, namely, 1) Before dexamethasone administration (control group), 2) after 2 days, 3) after 1 week, and 4) after 3 weeks of daily dexamethasone injections. Blood was taken from the rats on trisodium citrate (volume of citrate/volume of blood =1/9).

Assessment of Blood Coagulation (PT, APTT and FIB):

The ACL-200 automated coagulation system, Instrumentation Laboratory, was used to determine these parameters, using plasma obtained by centrifugation of the collected blood samples. The ACL is a nephelometric centrifugal analyzer which measures the intensity of light scattered by a plasma (sample) before, during and after clot formation.

- IL Test™ PT-EIB (97567-10): PT assay is sensitive to deficiencies in the activities of factor II, V, VII and X. The IL Test™ PT-Fibrinogen is a lyophilized extract from rabbit brain with the addition of optimal concentration of calcium ions.

- IL Test™ APTT (84687-10): APTT assay is sensitive to deficiencies in the activities of factor II, V, VII, IX, X, XI and XII. The intrinsic coagulation system is activated by micronized silica plus bovine brain cephalin, which is the substitute of platelet Factor 3. Clotting starts by the addition of calcium chloride.

Analysis of Results:

The final results are expressed as means ± S.E. for the number of separate experiments indicated. Statistical differences between dexamethasone-treated groups and control group were assessed by Student's *t* test, where $p < 0.05$ was considered significant and $p < 0.001$ was considered very highly significant.

Results

1- Control group: The mean values of PT and APTT were 11.9 ± 0.42 and 16.8 ± 0.17 seconds, respectively. The mean value of fibrinogen level in plasma (mg/100 ml) was 292.8 ± 7.33 (Table 1 and Fig. 1).

2- After 2 days of dexamethasone administration: As shown in Table 1 and Fig. 1, the mean values for PT and APTT were 11.3 ± 0.42 and 15.9 ± 5.51 seconds, respectively. Both values did not change significantly in comparison to the control group. The mean FIB level was 399.2 ± 8.65 mg/100 ml. In comparison with the control group, dexamethasone administration resulted in a very highly significant increase in FIB ($p < 0.001$).

3- After 1 week of dexamethasone administration: The mean value of PT was found to be 11.5 ± 0.38 seconds, which is insignificantly different from that of the control group. The mean APTT, however, was decreased to a very highly significant degree in comparison to controls ($p < 0.001$), reaching 12.3 ± 0.79 seconds. The mean FIB level, on the other hand, increased to 321.0 ± 7.29 mg/100 ml, which is a significant decrease in comparison to the control level ($p < 0.05$) (Table 1 and Fig. 1).

4- After 3 weeks of dexamethasone administration: Mean PT and APTT were 11.5 ± 0.38 and 11.9 ± 0.4 seconds, respectively. While PT was insignificantly different from the control group, APTT showed a very highly significant decrease ($p < 0.001$). Mean FIB at that time was 377.2 ± 10.21 mg/100 ml. This value is very highly significantly higher than in the control group ($p < 0.001$) (Table 1 and Fig. 1).

Table (1): Mean ± S.E. Values of Prothrombin Time (PT), Fibrinogen (FIB) and Activated Partial Thromboplastin Time (APTT) before (Control Group) and after 2 Days, 1 Week and 3 Weeks of Dexamethasone Administration (0.4 mg/Kg Body Weight Day) in Adult Rats (n=6).

Group of experiments	PT (seconds)	FIB (mg/100 ml)	APTT (seconds)
Control group	11.9 ± 0.42	292.8± 7.33	16.8 ± 0.17
After 2 days	11.3 ± 0.42	399.2 ± 8.65**	15.9 ± 0.51*
After 1 week	11.5 ± 0.36	321.0± 7.29*	12.3 ± 0.79**
After 3 weeks	11.5 ± 0.38	377.2± 10.21**	11.9 ±0.40**

* = Significant and ** = very highly significantly different from control group.

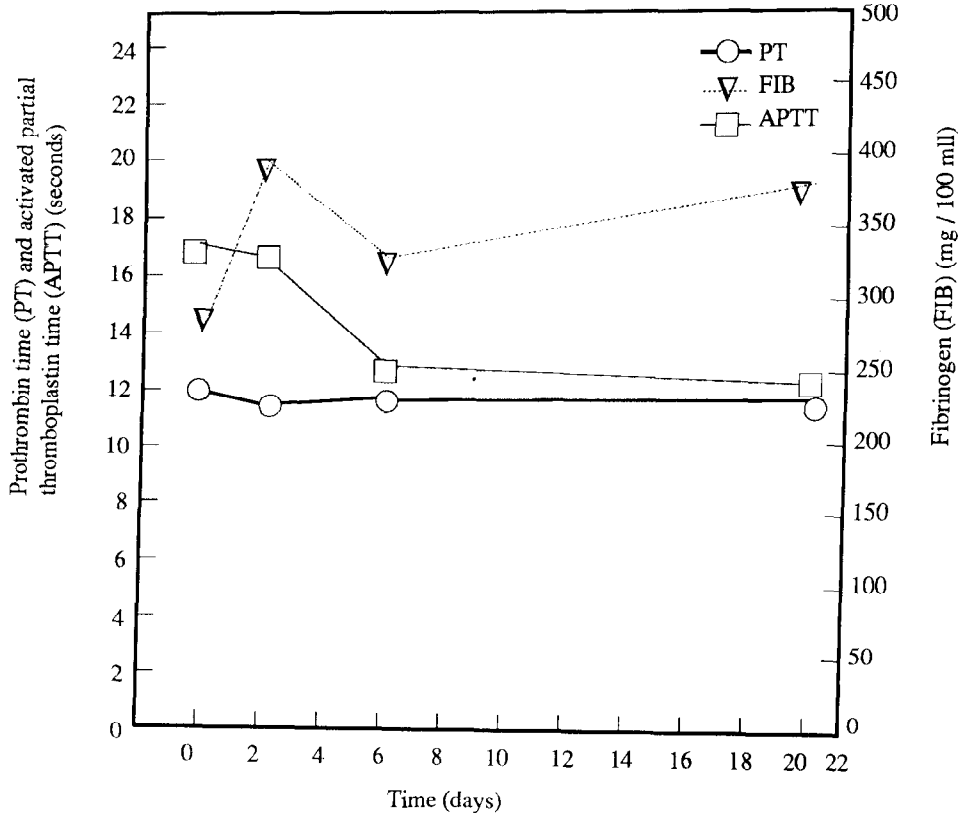

Fig. (1): Mean values of prothrombin time (PT), fibrinogen (FIB) and activated partial thromboplastin time (APTT) before and after 2 days, 1 week and 3 weeks of dexamethasone adminisration (0.4 mg/kg body weight) in adult male rats (n = 6).

Discussion

In the present study a continuous gradual decrease in APTT was observed, which was insignificant after 2 days, but became very highly significant already after one week of dexamethasone administration and even more so after 3 weeks. Although the values of APTT in the control group of the present study seem to be very low (mean APTT was 16.8 ± 0.17 seconds) in comparison to normal human APTT values (29 - 37 seconds), they were found to correspond totally with the results obtained by Emms and Lewis [8] in tats. These investigators also found a clear difference in the normal values of APTT, PT and fibrinogen between male and female rats. Therefore, only male rats were taken for the present study to avoid any sex-associated variations.

A decrease in APTT levels induced by glucocorticoid therapy was also observed by Jorgensen et al. [6]. Decreased APTT reflects an increased intrinsic coagulability. Increased factor VIII was reported in association with high glucocorticoid levels [9,10] and may, thus, be responsible for the clotting abnormalities found in such condition.

There is no significant change in PT throughout the 3 weeks during which dexamethasone was administered in the present study, which indicates that the extrinsic coagulation cascade is not affected by dexamethasone. This is in agreement with the results of a study, in which patients with Cushing's syndrome were examined [9]. An earlier investigation [6], however, described an increased PT in patients receiving prednisone. All the examined patients were suffering from collagen diseases, hematologic malignancies of other hypercoagulability states, which may be the reason for increased PT, despite glucocortioid therapy.

Fibrinogen, on the other hand, increased markedly 2 days after dexamethasone administration, became less after one week, but was still significantly higher than control level. After 3 weeks however, it increased further to a still higher peak.

Although these results correspond to those found by several investigators [10,13], they were not confirmed by a study, in which patients with autoimmune and malignant diseases, receiving corticosteroid therapy, were used [6], indicating that the underlying disease condition may interfere with the results and that an animal model may be preferable to study the effect of glucocorticoids per se on blood coagulation.

Our results are in agreement with an investigation in which long-term administration of glucocorticoids was found to decrease fibrinolysis and to sensitize to endotoxin-induced intravascular coagulation [12]. A more recent study [13] was carried out in patients who had undergone a standardized surgical trauma. Plasminogen activator activity was tested during the post-operative period, comparing a group receiving steroids with another receiving nonsteroidal agents. A decreased fibrinolytic activity, i.e. decreased plasminogen activator activity, was observed on the first and second post-operative days and after the first week in the group receiving high doses of corticosteroids, which was not the case in the nonsteroid group. The nonsteroid group showed decreased plasminogen activator activity only on the first postoperative day, which may have been a normal defense mechanism to the surgical stress.

Another study, which examined the ef-

fect of dexamethasone on protein synthesis by fibrosarcoma cells in vitro, found that it induces the formation of a new protein of 46 kD [14]. This effect could not be induced with female sex hormones and only to a very small degree with testosterone. Further purification and characterization of the 46 kD protein revealed that it was plasminogen activator inhibitor type I (PAI-1). The obtained protein also inhibited plasminogen activation. Thus, it seems likely that glucocorticoids regulate fibrinolysis in vivo by increasing the amount of plasminogen activator and preventing the activation of plasminogen to plasmin. Since plasmin lyses fibrin and fibrinogen [15] decreased plasmin would result in the accumulation of fibrinogen, which may explain the increased fibrinogen levels observed in our study.

To conclude, the present results confirm the stimulatory effect of glucocorticoids on the intrinsic coagulation cascade, an effect which may be due to increased factor VIII level. The extrinsic coagulation cascade, on the other hand, does not seem to be affected by glucocorticoid administration. In addition to the tendency for increased procoagulant activity, glucocorticoids result in a decrease in fibrinolytic activity, as indicated by increased fibrinogen levels. The decreased fibrinolysis may be attributed to increased PAI-1 formation, which inhibits plasmin synthesis and thus prevents the breakdown of fibrin and fibrinogen. Finally, it may be concluded that corticosteroids should only be administered with great caution in patients with underlying condition that favor intravascular coagulation, as they increase the imbalance towards a more thrombotic stage.

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