THE PLASMA CORTICOSTEROID PROFILE IN THE ADJUVANT INDUCED ARTHRITIC RAT MODEL

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1. Introduction

In 1963, Pearson [1] recorded his observations on adjuvant-induced arthritis (AIA) in rats. He was able to temporarily produce the clinical manifestations of rheumatoid arthritis by injecting Freund's complete adjuvant into the hind foot pads of rats. Similarities between human rheumatoid arthritis and the AIA rat model include the increase in red blood cell sedimentation [2,3], increase in fibrinogen levels [2,4], increase in α_2 and γ globulins with decrease in albumin [5,6], presence of the RF factor in sera [7,8], and the shift in the normal circadian rhythm of corticosteroids [9,10].

Margraf et al. in 1972 [11] reported a significant increase in cortisol 21-acetate levels in the plasma of rheumatoid patients. Because of the widespread use of the AIA rat model in arthritis research, this study was undertaken to determine if the corticosteroid profile of the AIA rat model was similar to that of rheumatoid arthritis in man. The results show a minimization of corticosterone, a 5 day periodicity in the cortisol levels and the absence of the 21-acetates of cortisol and corticosterone. This profile does not correspond to that reported for rheumatoid arthritis.

2. Experimental

Two independent groups of COBS, CD, outbred male rats (400–700 g) on a diet of Purina Lab Chow were artificially induced by intradermal injection of 0.1 ml of Freund's complete adjuvant into the plantar surface of the hind foot pad. Animals within a group had a common induction date and were sacrificed at different periods up to 35 days after induction. Control animals were regularly monitored throughout this study. The rats were decapitated between 9:00–11:00 h and ~5 ml of whole blood was collected in a vessel containing 30 mg heparin and 50 μg phenylmethyl sulfonyl fluoride. The phenylmethyl sulfonyl fluoride was used to prevent deesterification of the 21-acetates. The whole blood was immediately divided in half and 250 ng corticosteroids (cortisol, corticosterone, cortisol 21-acetate and corticosterone 21-acetate) were added to 1 portion to establish their stability during the analytical procedures. This standard addition portion was analyzed simultaneously with the other, non-standard addition portion. The plasma obtained by centrifugation was quickly extracted by shaking for 7 min with 8 parts of cold methylene chloride. Cold 0.1 N NaOH (1 ml) was added and the solution reshaken for 30 s. The methylene chloride layer was filtered using a 0.5 μm filter and taken to dryness under nitrogen. The sample was reconstituted in 100 μl methanol for application to the column.

A Waters ALC-200 liquid chromatograph (Waters Associates, Milford, MA) equipped with a model 440, 254 nm fixed wavelength UV detector was used throughout this study. Complete separation of the corticosteroids of interest was obtained at ambient temperatures using a mobile phase of acetonitrile–water (35:65, v/v) with a Waters, 30 cm, μ-Bondapak ODS-C_{18} column at a 1.5 ml/min flow rate.

3. Results

Maximum swelling in the hind foot of the induced rats was observed ~14 days after induction and this was paralleled by changes in 2 of the corticosteroids. Corticosterone showed a minimization between days 10–17 (fig.1, table 1) while cortisol showed a periodicity between days 7–22 (fig.2). The cortisol perio-

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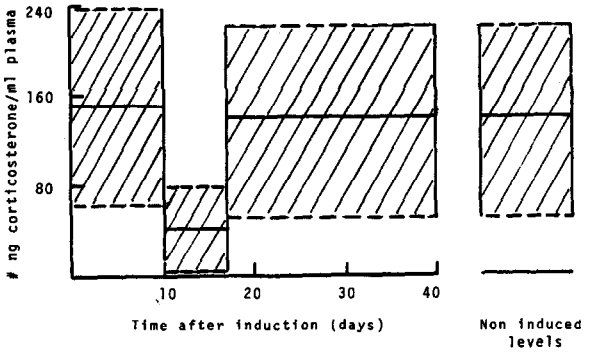

Fig.1. Mean corticosterone levels of AIA-induced rats and control rats. Error ranges are ±1 SD.

dicity was found reproducible in 2 independent groups of AIA rats. The level of cortisol was negligible in the non-induced rats and also in the AIA rats except during the 7–22 day period (fig.3, table 1). No cortisol 21-acetate or corticosterone 21-acetate was detected above the instrumental sensitivity of 1 ng/ml plasma.

4. Discussion

Corticosteroids, such as cortisol and cortisone, have been used for the control of inflammation for many years. The action of these corticosteroids seems in part to depend upon their ability to stabilize cell membranes [12]. The low level of corticosterone in AIA rat plasma may in some way reflect the observed inflammatory response.

The increase in cortisol levels showing a 5 day periodicity did not correlate with such experimental parameters as body weight, circadian rhythm, feeding

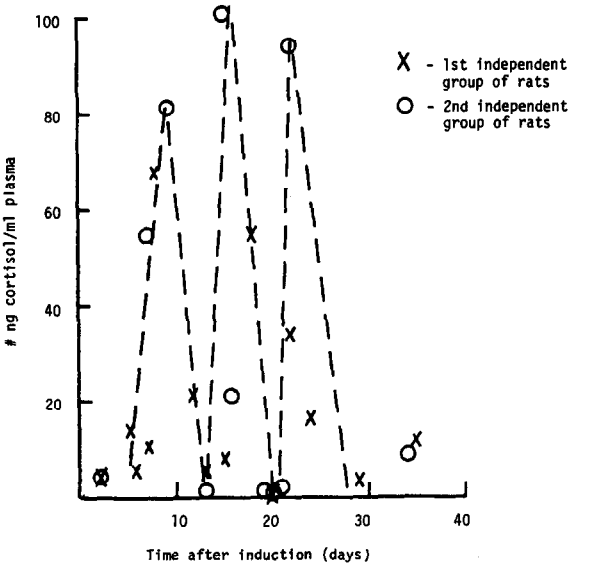

Fig.2. Cortisol periodicity between days 7–22 after induction.

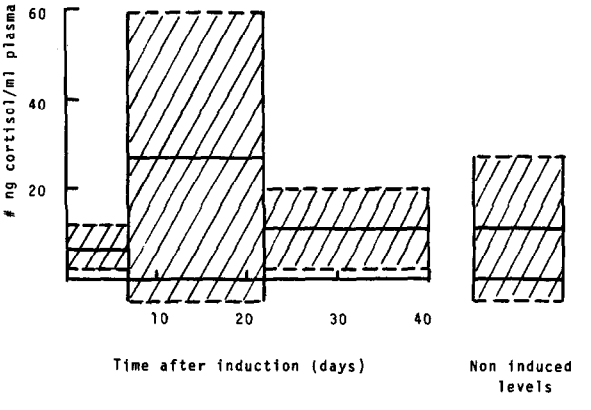

Fig.3. Mean cortisol levels of AIA-induced rats and control rats. Error ranges are ±1 SD.

Table 1
Corticosteroid levels in AIA rats

Cortisol		Corticosterone	
Period after induction	Ng corticosteroid /ml plasma[a]	Period after induction	Ng corticosteroid /ml plasma[a]
0– 6 days	7 ± 5 (4)	0– 9 days	150 ± 87 (8)
7–22 days	27 ± 32 (23)	10–17 days	43 ± 36 (8)
23–35 days	11 ± 9 (8)	18–35 days	136 ± 83 (19)
Concentration of control rats (non-induced) 11 ± 16 (13)		Concentration of control rats (non-induced) 135 ± 85 (13)	

[a] Mean concentration ± SD (no. data points)

time or light–dark cycles. There is no explanation for this cortisol periodicity, however, it is well established that some steroids, i.e., 20-α-dihydroprogesterone and 17-hydroxypregnenolone, follow a 5 day circatrigentan variation (estrus cycle) in rats [13].

The main feature of the corticosteroid profile was the absence of the corticosteroid 21-acetates. This indicated that the AIA rat model is not similar to human rheumatoid arthritis in respect to the corticosteroid profile. The application of our HPLC analysis method to human arthritic plasma has provided preliminary results showing the enhancement of cortisol 21-acetate levels as in [11].

The lack of correlation between corticosteroid profiles in man and AIA rats may be due to the artificial induction, i.e., a short-term, temporary, inflammatory response as compared to the long-term state in rheumatoid arthritic individuals. The fact remains that one should be aware of these plasma corticosteroid profile differences when using AIA rats for arthritis studies.

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