HAEMATOBIOCHEMICAL CHANGES IN DRUG INDUCED IMMUNOSUPPRESSED RABBITS.

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ABSTRACT

Haematobiochemical study was carried out in Dexamethasone and Cyclosporine A induced immunosuppressed rabbits. The results reveals that the drugs induced both negative and positive effect on haematological profile . Hemoglobin, TLC, protein, sodium and cortisol level significantly whereas glucose, cholesterol, potassium levels and alkaline phosphatase activity significantly increased .

KEY WORDS: **Dexamethasone, Cyclosporine A, Immuno-suppression, Haematobiochemical, Rabbits.**

INTRODUCTION

Immuno-suppression is a negative control or regulation of immunologic reactivity. Corticosteroids are the most widely used agents in the treatment of inflammatory and immunity mediated diseases. But their hazards and side-effects are numerous and must always be considered when embarking on therapeutic regimens.

Cyclosporin A (CsA) is capable of suppressing the development of humoral and cell-mediated immune responses in contrast to other immunosuppressive drugs, which destroy many types of cells within lymphoid compartment. The primary action of CsA is against thymus derived (T) lymphocytes and is reversible (Nickell *et. al.,1982*). Dexamethason is more potent in suppressing the cell mediated immunity than the humoral immunity.

The present study is undertaken taken to inestigate haematobiochemical alterations in dexamethasone and CsA induced immunosuppressed rabbits.

MATERIALS & METHODS

The experiment was conducted on 24 New-Zealand white rabbits of both sexes having body weight between 1000-1050 gm animals were selected randomly and divided into four groups namely Group A, Group B, Group C_1 (control for Group A) and Group C_2 (control for Group B). Group A was injected subcutaneously with Cyclosporine A at the dose rate of 15mg/Kg body weight with olive oil as vehicle, for 21 days. Group B was injected intramuscularly with Dexamethasone at the dose rate of 2 mg/kg body weight for 21 days. Control group C_1 was injected with the olive oil vehicle for 21 days and C_2 was kept untreated control. Blood was collected on 0 day before the commencement of the treatment followed by 8th ,15th and 22nds day . All the Haematological and Biochemical parameters were estimated following standard methods (King and King 1954) . The serum cortisol level was estimated by ELISA method (DRG cortisol ELISA EIA-1887). The data were analyzed statistically (Snedecor & Cochran, 1970).

RESULTS AND DISCUSSION

The results presented in table 1, reveals that the Haemoglobin level, TLC, , serum total protein, serum albumin, serum globulin, serum sodium and cortisol decreased significantly whereas blood glucose , serum cholesterol, serum ALP activity, serum potassium showed a significant (P=0.01) increase in both groups A and B when compared with their respective control groups.

A decrease in Hb level observed on 8^{th}, 15^{th} and 22^{nd} day of treatment in the group A can be attributed to the toxic effect produced by prolonged Cyclosporine A (CsA) therapy. CsA produces haemolytic uremic syndrome and chronic nephrotoxicosis. The increase in Hb level noticed on 8^{th}, 15^{th} and 22^{nd} day of treatment in group B may be attributed to increase in serum iron level following corticosteroid treatment. Kaneko *et al.* (1997) stated that corticosteroid administration increases dramatically serum iron in horses and dogs. This increase in serum iron may lead to increased haem synthesis consequently increases the synthesis of Hb. These findings are in agreement with Bogan *et al*. (1983) who stated that glucocorticoids tend to cause

an increase in Hb level and an increase in the number of circulating erythrocyte also.

Hyperglycemia observed on 8th, 15th and 22nd day of treatment in both the group A and B as compared to control group C may be attributed the glucogenic effect and inhibition of glucose uptake in peripheral tissues produced by dexamethasone and CsA. Our findings are in agreement with Schonfeld *et al.* (1997) who stated that the CsA treated rats developed hyperglycemia on day 9 of the treatment which was attenuated by Silibin, a plant extract with antioxidant and membrane stabilizing properties. Puvadolpirod and Thaxton (2000) reported similar finding in chicken treated with ACTH, however Huff *et al.* (2001) reported a decrease in serum glucose level in turkeys treated with dexamethasone which is contradictory to our findings.

Hypoproteinemia and hypoalbuminemia noticed on 8th, 15th and 22nd day of treatment in both group A and B might be attributed to the protein catabolism produced by these drugs. Glucocorticoid enhances the protein catabolism and inhibits the synthesis of protein. Hypoproteinemia produced by CsA may also be attributed to the hepatotoxicity leading to liver insufficiency and consequently reduced synthesis of protein.

Hypoglobulinemia in group A observed on 8th, 15th and 22nd day of treatment may be due to the direct effect of CsA on the immune system leading to reduction in gamma globulin, which contributed to the hypoglobulinemia.

Hypercholesterolemia observed on 8th, 15th, 22nd day of treatment in the group A may be attributed to cholestasis produced by CsA which leads to increased cholesterol level (Kaneko *et al.* 1997; Chanussol and Benkoel 2003). On prolonged therapy Dexamethasone might have produced adrenocortical insufficiency leading to reduced cortisol synthesis. Since cholesterol is the precursor for the synthesis of steroids, in the absence of cortisol synthesis cholesterol level is increased in group B.

Hyponatremia and hyperkalemia noticed on 8th, 15th, 22nd day of treatment in group A may be due to the nephrotoxicity of CsA. The hyponatremia noticed on 15th and 22nd day of treatment may be due to the withdrawal of dexamethasone therapy leading to adrenal insufficiency. Hyperkalemia noticed on 8th, 15th, 22nd day of treatment in group A may be attributed to the nephrotoxicity of CsA. Hypokalemia noticed on 8th day of treatment in group B may be attributed to the minimal mineralocorticoid effect possessed by dexamethasone. Bogan *et al.* (1983) reported that glucocorticoids enhance the potassium excretion, which is a more consistent effect than sodium retention, and prolonged administration of high dose can result in hypokalemia. Swaminathan (2004) stated that the common cause of adrenal hypo-function is the therapeutic use of glucocorticoids leading to suppression of the pituitary adrenal axis.

Increased urea & BUN level on 8th day of treatment in group A may be attributed to the perennial azotemia due to the hepatotoxicity produced by CsA (Kaneko *et al.*, 1997). On 15th and 22nd day of treatment the urea & BUN level gradually reached their normal baseline value in group A. This trend may be due to the increased fractional excretion of urea and BUN influenced by polyurea produced due to the renal failure, attributed to the nephrotoxicity of CsA (Kaneko *et al*., 1997). This finding is in agreement with the findings of Latimer *et al.* (1986) who reported increased serum urea concentration in cats administered with CsA. The increase in urea & BUN level on 8th day of treatment in group B may be attributed to the catabolic effect of dexamethasone on protein. On 15th and 22nd day of treatment urea & BUN level gradually returned to normal baseline value in group B, which may be due to reduced protein catabolism due to withdrawal of dexamethasone therapy.

Elevated ALP activity on 8th, 15th, 22nd day of treatment in group A may be due to cholestasis produced by CsA. Chanussol and Benkoel (2003) reported intrahepatic cholestasis in rats treated with CsA. Kaneko *et al.* (1997) reported that cholestasis induces hepatic ALP. Intrahepatic cholestasis tends to cause progressive increase in ALP activity. Increase in serum ALP precedes hyperbilirubinemia. The elevated ALP activity on 8th day of treatment in group B may be due to the Iatrogenic Cushing's syndrome, produced by continuous dexamethasone therapy (Swaminathan, 2004).

But the persistence of elevated ALP levels on 15th and 22nd day of treatment even after the withdrawal of dexamethasone therapy may be due to osteoporosis. Osteoporosis is a characteristic effect produced in Iatrogenic Cushing's syndrome that occurred before the withdrawal of therapy (Swaminathan, 2004).

Table : Hematological and Biochemical Changes in Drug Induced Immunosuppressed Rabbits.

Parameters	Days	Animal Groups			
		A	B	C_1	C_2
Haemoglobin (gm/dl)	0 day(PT)	9.04±0.62	9.24±0.30	9.48±0.02	9.45±0.02
	8th Day	4.40±0.07	14.18±0.03	9.46±0.03	9.48±0.02
	15th Day	3.68±0.66	12.32±0.08	9.50±0.02	9.41±0.03
	22nd Day	4.39±0.07	11.14±0.11	9.47±0.04	9.35±0.02
TLC ($\times 10^3$/c. mm.)	0 day(PT)	6.73±0.12	6.67±0.2	6.28±0.11	6.25±0.12
	8th Day	5.31±0.012	5.23±0.015	6.25±0.08	6.24±0.08
	15th Day	505±0.07	5.41±0.034	6.3±0.1	6.35±0.1
	22nd Day	5.47±0.012	5.41±0.012	6.28±0.14	6.31±0.12
Serum Glucose (mg/dl)	0 day(PT)	92.28 ±0.43	92.36 ±0.46	92.83 ±0.45	92.45 ±0.46
	8th Day	112.2 ±0.84	111.44 ±0.99	93.21 ±0.3	92.56 ±0.46
	15th Day	107.77 ±0.96	106.35 ±0.89	93.15 ±0.28	92.77 ±0.43
	22nd Day	101.54 ±0.37	101.01 ±0.22	93.91 ±0.2	92.56 ±045
Serum Total protein (g/dl)	0 day(PT)	7.25 ±0.02	7.27 ±0.04	7.4 ±0.08	7.3 ±0.02
	8th Day	6.51 ±0.05	6.51 ±0.06	7.28 ±0.04	7.4 ±0.04
	15th Day	6.43 ±0.03	6.43 ±0.03	7.3 ±0.04	7.35 ±0.03
	22nd Day	5.88 ±0.04	6.44 ±0.03	7.27 ±0.03	7.31 ±0.04
Serum albumin (g/dl)	0 day(PT)	4.54 ±0.03	4.56 ±0.03	4.51 ±0.04	4.51 ±0.03
	8th Day	4.17 ±0.04	4.2 ±0.05	4.51 ±0.04	4.52 ±0.04
	15th Day	4.13 ±0.03	4.1 ±0.04	4.49 ±0.04	4.56 ±0.04
	22nd Day	4.08 ±0.03	4.14 ±0.03	4.51 ±0.04	4.57 ±0.03
Serum globulin (g/dl)	0 day(PT)	2.71 ±0.03	2.71 ±0.04	2.78 ±0.06	2.8 ±0.05
	8th Day	2.35 ±0.02	2.31 ±0.00	2.77 ±0.05	2.76 ±0.06
	15th Day	2.31 ±0.00	2.32 ±0.00	2.83 ±0.05	2.79 ±0.05
	22nd Day	1.78 ±0.05	2.3 ±0.01	2.74 ±0.05	2.74 ±0.05
Serum cholesterol (mg/dl)	0 day(PT)	51.14 ±0.45	52.43 ±0.43	51.64 ±0.56	50.91 ±0.45
	8th Day	70.37 ±2.23	72.23 ±1.0	51.09 ±0.44	51.2 ±0.44
	15th Day	91.78 ±0.38	92.59 ±0.52	51.49 ±0.41	51.59 ±0.43
	22nd Day	70.36 ±2.21	73.36 ±0.88	52.71 ±0.4	51.76 ±0.4
Serum sodium (mg/dl)	0 day(PT)	126.1 ±0.29	126.01 ±0.33	126.19 ±0.3	127.1 ±0.3
	8th Day	108.65 ±1.23	151.88 ±0.47	126.22 ±0.28	126.56 ±0.25
	15th Day	108.5 ±1.43	112.06 ±0.56	126.22 ±0.22	125.7 ±0.22
	22nd Day	107.11 ±1.37	112.27 ±0.88	126.18 ±0.09	126.12 ±0.29
Serum potassium (mg/dl)	0 day(PT)	4.22 ±0.07	4.32 ±0.03	4.38 ±0.02	4.35 ±0.02
	8th Day	6.29 ±0.1	2.41 ±0.08	4.3 ±0.06	4.21 ±0.07
	15th Day	6.23 ±0.1	6.34 ±0.06	4.32 ±0.03	4.35 ±0.06
	22nd Day	6.29 ±0.06	6.41 ±0.04	4.33 ±0.03	4.32 ±0.03
BUN (mg/dl)	0 day(PT)	14.33 ±0.15	14.37±0.16	14.03 ±0.02	14.06 ±0.02
	8th Day	19.62 ±0.17	18.77 ±0.26	14.49 ±0.17	14.27 ±0.15
	15th Day	14.54 ±0.2	14.07 ±0.11	14.55 ±0.14	14.34 ±0.02
	22nd Day	13.99 ±0.04	13.34 ±0.15	14.24 ±0.16	14.17 ±0.03
Serum urea (mg/dl)	0 day(PT)	30.68 ±.033	30.77 ±0.34	30.05 ±0.03	30.11 ±0.03
	8th Day	42.02 ±0.37	40.13 ±0.6	31.05 ±0.37	30.56 ±0.33
	15th Day	31.13 ±0.43	30.1 ±0.2	31.17 ±0.31	30.71 ±0.32
	22nd Day	29.95 ±0.1	30.7 ±0.32	30.67 ±0.33	30.35 ±0.34
ALP (mg/dl)	0 day(PT)	6.06 ±0.04	6.06 ±0.04	5.93 ±0.1	5.91 ±0.02
	8th Day	8.78 ±0.17	8.96 ±0.09	5.97 ±0.02	6.1 ±0.1
	15th Day	9.16 ±0.05	9.12 ±0.05	6.1 ±0.04	6.07 ±0.04
	22nd Day	9.18 ±0.05	9.13 ±0.03	6.05 ±0.06	5.99 ±0.04
Cortisol (ng/dl)	0 day(PT)	1200 ±68.1	1260±80.18	1290±127.28	1200±68.4
	8th Day	466.67±24.72	2.5±1.71	1200±68.31	1250±80.18
	15th Day	458.33±23.86	266.67±24.72	1266.67±80.28	1290±125.13

The decreased cortisol level in group A on 8th and 15th day of treatment may be due to inhibition of the production of certain cytokines specially IL-2 by suppression of calinurin stimulated events and also by the increased expression of TGF-â.

The decrease in cortisol concentration on 8th and 15th day of treatment in group B may be due to the negative feed back inhibition of synthesis of the endogenous cortisol through Hypothalamo-pituitary-adrenal axis.

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