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THE EFFECT OF LONG TERM CORTICOSTEROID ADMINISTRATION ON LIPID AND PROSTAGLANDIN LEVELS

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SUMMARY

New Zealand white rabbits were given one of two forms of methylprednisolone (Depo-Medrol or Solu-Medrol) for sixteen weeks. The administration of these corticosteroids resulted in elevated total lipids, triglycerides, cholesterol and free fatty acids by the end of one week of treatment. Plasma prostaglandin levels (combined PGA and PGE) were also found to be elevated. Corticosteroids may initially reduce prostaglandin levels but the alterations in lipid metabolism which can lead to elevated prostaglandin precursors eventually appear to result in elevated levels. It is suggested that the alterations in prostaglandin levels may play a role in the development of the vascular occlusion leading to bone necrosis (avascular necrosis), a frequent complication of long term corticosteroid therapy.

INTRODUCTION

The administration of corticosteroids produces alterations in the distribution of fat deposits from peripheral to central stores and is accompanied by pronounced hyperlipidemia. Elevated levels of triglycerides, cholesterol, phospholipids and total lipids as well as total and free fatty acid have been demonstrated following corticosteroid treatment [1–4].

There have been conflicting reports concerning the effects of corticosteroids on prostaglandin production. Several investigators concluded that such steroids had little influence on prostaglandin levels [5–9]. More recent reports have indicated that in cell culture corticosteroids reduce prostaglandin output [10–14]. In this report the administration of methylprednisolone to rabbits for sixteen weeks was accompanied by elevations in serum lipid levels as well as elevations in plasma prostaglandin levels.

This study was undertaken to establish the effects of long term corticosteroids on the production of prostaglandins in an *in vivo* system and to elucidate the mechanism of development of the bone disorder, avascular necrosis, which is a frequent complication of long term corticosteroid administration. A possible role for prostaglandins in the etiology of this bone lesion is suggested.

METHODS

Daily intramuscular injections of methylprednisolone were given to two groups of 3–4 kg New Zealand white rabbits. A group of eight rabbits was given 0.80 mg of Depo-Medrol (an aqueous suspension of methylprednisolone acetate). A second group of six rabbits was given 0.80 mg of Solu-Medrol (an aqueous solution of methylprednisolone sodium succinate). At week five the dose of Depo-Medrol was changed to one injection every third day to reduce morbidity. Administration of steroids greatly increases susceptibility to infection. Therefore, both groups were also given daily prophylactic intramuscular injections of 37.5 mg of Ancef (cefazolin sodium) a broad range antibiotic. A third group of four rabbits was given no medication, was fed a standard vivarium diet and was allowed to eat and drink *ad libitum*. A fourth group of six rabbits was given antibiotic but no steroid and was placed on a reduced dietary intake to simulate the weight loss expected in the experimental groups.

Serum and plasma from animals in each group were collected at the end of weeks 1, 2, 3, 5, 8, 11 and 16. Serum was analyzed for total lipids [15], cholesterol [16], triglycerides [17] and free fatty acids [18]. Prostaglandins in 0.125 ml of plasma was extracted with two- 1.0 ml vols of ethyl acetate. The organic phases were combined, dried under nitrogen and redissolved in Tris–HCl buffer pH 7.4 containing 1 mg/ml gelatin. Prostaglandin was measured as PGB, after conversion of PGA and PGE to PGB by alkaline treatment of the plasma extracts, by radioimmunoassay [19]. An extraction efficiency of 85–90% was determined by adding a small quantity of [^{3}H]-PGB to plasma samples before treatment.

The possibility that the elevated blood lipids, particularly free fatty acids, were interfering with the prostaglandin determinations was investigated by separating out the prostaglandins in sample plasma extracts by chromatography on silicic acid. Lipids were first extracted from 0.40 ml of plasma with ethyl acetate and then applied to a short column of silicic acid (Unisil), previously washed with heptane.

Table 1. Serum lipid levels following corticosteroid administration

		Triglycerides	Cholesterol	Total lipid
Depo-medrol	(17)	905 ± 178	573 ± 137	2046 ± 661
Solu-medrol	(15)	1068 ± 196	583 ± 99	2725 ± 653
Restricted diet	(15)	230 ± 26	198 ± 32	396 ± 46
Unrestricted diet	(11)	276 ± 33	149 ± 22	324 ± 43

Concentrations are in mg per 100 ml and are expressed as means ± S.D. of mean of serum lipid levels measured at intervals over the sixteen week course of the experiment. The number of samples analyzed are in parenthesis. The differences in lipid levels in the corticosteroid treated and untreated groups were found to be statistically significant ($P < 0.05$).

The column was then washed with ether and the eluate collected. Prostaglandins were then eluted from the column by washing with 2% methanol in ethyl acetate. Both fractions were evaporated to dryness, the residues redissolved in Tris–HCl buffer and analyzed for prostaglandins as described above.

RESULTS

Over the sixteen week course of this study the rabbits given Depo-Medrol lost an average of 80 g per week. Those given Solu-Medrol and those on the restricted diet had slight weekly weight losses. The rabbits on a normal diet gained approximately 40 g per week.

Statistically significant elevations ($P < 0.05$) in serum triglyceride, cholesterol, and total lipids were found in the treated rabbits compared to controls by the end of the first week. Subsequent serum lipid measurements demonstrated that these levels remained elevated for the remainder of the experiment. No significant differences were observed in the lipid concentrations of the two control groups. Table 1 shows the average of the measurements of each lipid taken during this study. These results are in accord with previous reports of lipid levels following corticosteroid administration [1–4].

Experimental groups also exhibited statistically significant elevations ($P < 0.05$) in levels of serum free fatty acids and plasma prostaglandins. The concentration of each of these groups of lipids increased during the early weeks and then remained 2–3 fold higher than the corresponding levels in control rabbits (Fig. 1). The average level of free fatty acid and prostaglandin calculated from the measurements made of each over the course of the experiment is shown in Table 2. Steroid treated groups had serum free fatty acid levels of 2.82 and 2.79 meq/l while concentrations of 1.17 and 1.19 meq/l were found in the untreated rabbits on restricted and unrestricted diets respectively. Prostaglandin concentration measured as PGB, was 5.85 and 4.73 ng/ml in the treated groups and 2.24 and 2.43 ng/ml in the control groups. The parallel increases in the concentrations of plasma prostaglandin and serum free fatty acids in the experimental groups were consistent with the possibility that the elevations in prostaglandin levels was a direct result of the elevations in free fatty acids.

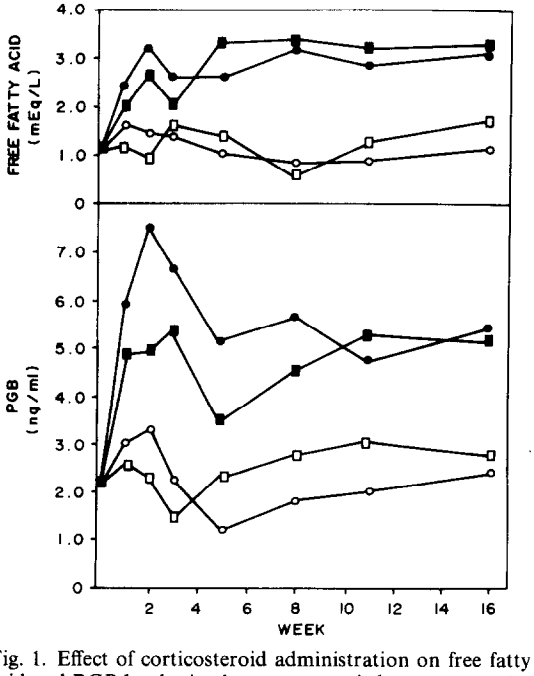

Fig. 1. Effect of corticosteroid administration on free fatty acid and PGB levels. Analyses were carried out on samples from rabbits given Depo-Medrol (●) and Solu-Medrol (■) and control rabbits on restricted (○) and normal (□) diets. Concentrations at start of experiment were the average blood levels of all groups prior to steroid injection.

Table 2. Serum free fatty acid and plasma prostaglandin levels after corticosteroid administration

		Free fatty acid (meq/l)	Prostaglandin (ng/ml)
Depo-medrol	(17)	2.82 ± 0.13	5.85 ± 0.37
Solu-medrol	(15)	2.79 ± 0.23	4.73 ± 0.27
Restricted diet	(15)	1.17 ± 0.14	2.24 ± 0.26
Unrestricted diet	(11)	1.19 ± 0.19	2.43 ± 0.21

Concentrations are means and S.D. of mean of free fatty acid and prostaglandin levels measured at intervals over the sixteen week course of the experiment. The number of samples analyzed are in parenthesis. The differences in both free fatty acids and prostaglandins in the corticosteroid treated and untreated groups were found to be statistically significant ($P < 0.05$).

Table 3. Distribution of plasma prostaglandin following silicic acid chromatography

		Prostaglandin in ether fraction†	Prostaglandin in ethyl acetate/methanol fraction†	Prostaglandin in unchromatographed plasma‡
Depo-medrol	(5)	192 ± 29	845 ± 43	648 ± 38
Solu-medrol	(3)	166 ± 27	1040 ± 54	727 ± 44
Restricted diet	(5)	105 ± 27	580 ± 36	394 ± 32

Means and S.D. of mean of prostaglandin measured as pg of PGB in plasma collected on week 11. The number of samples analyzed are in parenthesis. † Ethyl acetate extracts of 0.40 ml of plasma were applied to silicic acid columns which were then washed with ether followed by 2% methanol in ethyl acetate. Prostaglandin was determined in each wash. ‡ Prostaglandin in ethyl acetate extracts of 0.20 ml of plasma. Corrected for an 85% extraction efficiency.

Prostaglandins in plasma were also measured following separation from free fatty acids and other lipids by silicic acid chromatography. The results, tabulated in Table 3, show that the non-prostaglandin lipid fraction (ether fraction) did contain material detectable as PGB. Analysis of the prostaglandin fraction (ethyl acetate/methanol fraction) demonstrated that statistically significant elevations ($P < 0.05$) in prostaglandin levels could still be observed after removal of lipid material which could potentially interfere with the radioimmunoassay determinations.

DISCUSSION

Corticosteroid administration increases lipase activity and thereby elevates serum free fatty acid levels. It would be expected therefore that arachidonic acid was liberated. Studies of *in vitro* systems have shown that the concentration of arachidonic acid is a rate limiting factor in PGE biosynthesis [20]. Promotion of the availability of this precursor *in vivo* therefore could likely lead to elevations of prostaglandin production. Studies in cell culture [21] and an *in vivo* study in dogs [22] have substantiated that exogenously supplied fatty acid precursor stimulates the production of prostaglandin to levels proportional to the concentration of the fatty acid. The findings of similar elevations of free fatty acid and prostaglandin levels following steroid administration in this report are consistent with these observations.

There is considerable controversy concerning the effects of corticosteroids on prostaglandin production. In cell-free homogenates, Vane reported very little influence of such compounds on prostaglandin output [5]. Lewis and Piper showed that corticosteroids could inhibit prostaglandin mediated vasodilation but did not prevent their *in vivo* formation [6]. There have been reports that corticosteroids are ineffective in reducing prostaglandin production in inflammatory exudates [7], in perfused rat spleen [8] and in platelets [9]. whereas considerable reductions of prostaglandin were observed in the presence of non-steroid anti-inflammatory drugs.

More recently there have been reports that corticosteroids can reduce prostaglandin production *in vitro* through an effect on precursor availability. For instance, such steroids inhibited norepinephrine induced release of PGE but the inhibition was overcome by infusion of arachidonic acid [10]. Corticosteroids have also been reported to be capable of suppressing prostaglandin accumulation in the culture media of rheumatoid synovia [11] and mouse fibrosarcoma cells [12]. Hong and Levine[13] demonstrated that steroids inhibited the release of arachidonic acid in these *in vitro* situations but did not inhibit the production of prostaglandin from exogenously supplied precursor. Similar results were obtained by Floman and Zor[14] in an *in vitro* examination of inflamed synovia. They noted, however, that the concentration of steroid used exceeded the therapeutic plasma levels in humans and that lower concentrations of steroid were ineffective.

The first suggestion that methylprednisolone produces an increase in prostaglandin production was made by Glenn *et al.*[23]. In that study, the administration of this steroid to rats produced slight decreases in serum $PGF_{2\alpha}$ concentrations at low doses but elevated them at higher doses. Increased plasma $PGF_{2\alpha}$ levels have also been observed in rabbits following long term corticosteroid administration in preliminary experiments carried out in our laboratory.

Several possibilities exist which may explain these conflicting results. One explanation may be that the *in vivo* and *in vitro* responses to corticosteroids are different. Such an effect has been observed with sodium salicylate, which frequently has little effect on prostaglandin production *in vitro* but can markedly affect their output *in vivo* [24]. A more likely explanation may be that steroid administration results in an initial decrease in prostaglandin levels, followed by an increase in their production as the alterations in lipid metabolism, including liberation of prostaglandin precursors, become manifest. This may also be linked in some manner to the dose of steroid utilized. A third possibility may be that all corticosteroids do not elicit the same effect on prostaglandin output. The methylprednisolone effect therefore, may be a response that is not shared with other corticosteroids.

A frequent complication of long term corticosteroid administration is avascular necrosis of bone. This disorder is characterized by areas of bone death as a consequence of vascular obstruction in the subchondral bone of large joints. This results in a painful joint requiring surgical reconstruction. It is a major problem in individuals receiving long term corticosteroid treatment (frequently methylprednisolone) for immunosuppression following successful renal transplantation. The etiology of the vascular occlusion which leads to this bone complication is unresolved. Several of the side effects of corticosteroid therapy have been implicated in the development of this disorder including systemic fat embolism, osteoporosis and vasculitis [3, 25 27]. However, no mechanism has as yet been described which can adequately account for this phenomenon. It may be necessary to take into account a combination of several factors in order to understand this malady. The observation of elevated prostaglandin levels in this report suggests that prostaglandin may also play a role in this process. Potential modes of such interactions may include participation in the development of local inflammation of the blood vessels (vasculitis) in bone tissue or a possible interaction with the circulating fat emboli contributing to the lodging of an emboli in the vasculature.

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