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Abstract
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Disciplines
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Effects of In Vivo Dexamethasone Administration on In Vitro Bovine Polymorphonuclear Leukocyte Function

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Polymorphonuclear leukocyte function was evaluated in vitro after in vivo administration of a single dose of dexamethasone to cattle. Purified polymorphonuclear leukocytes from dexamethasone-treated cattle displayed enhanced random migration under agarose but impaired ingestion of Staphylococcus aureus, Nitro Blue Tetrazolium reduction, chemiluminescence, iodination, and antibody-dependent, cell-mediated cytotoxicity. The depression of iodination may have been related to a drop in the proportion of eosinophils present in the polymorphonuclear leukocyte preparations after dexamethasone administration.

Glucocorticoids, when administered to humans or animals, have been observed to decrease the inflammatory response and to predispose to bacterial infection (9). Polymorphonuclear leukocytes (PMNs) play an important role in both the inflammatory response and the control of bacterial infection. For this reason, numerous investigations have been conducted on the effects of glucocorticoids on PMN function. There are many reports of defects in PMN function after in vitro or in vivo exposure to glucocorticoids. However, it is difficult to reach a conclusion on the overall effects of glucocorticoid therapy on PMN function owing to conflicting results and variations in experimental techniques. One potential reason for conflicting results when PMNs are exposed in vitro to glucocorticoids is that, in some experiments, the succinate form of glucocorticoids was used (8, 13, 17). The succinate must be split off in the liver by an esterase before the drug is active. Spontaneous cleavage of the succinate under in vitro conditions occurs slowly. With extremely high concentrations of the succinate compound enough free glucocorticoid may be present to affect PMN function (3, 19, 21). Another possible explanation for the presence of conflicting results when comparing the in vitro and in vivo exposure of PMNs to glucocorticoids is that in vivo administration of glucocorticoids may have indirect effects on PMN function by altering the activity of other mediators of inflammation. For example, betamethasone has been shown to block prostacyclin synthesis by vascular endothelial cells (16). Prostacyclins may have potent effects on PMN function (31). For these reasons, it is desirable to design experiments with in vivo treatment of healthy individuals with glucocorticoids to obtain information relative to the effects of therapeutic glucocorticoid administration on PMN function.

Some of the reports of in vivo treatment with glucocorticoids have compared PMNs from patients who are on glucocorticoid therapy for an underlying pathological process to PMNs obtained from normal individuals (3, 4, 27). It is difficult to be certain whether the defects in PMN function observed are due to glucocorticoid treatment or to the underlying pathologic process. There are reports comparing the function of PMNs from normal subjects who have voluntarily received a therapeutic dosage of glucocorticoid to that of normal controls (6, 18). This has yielded important information, but only one or two aspects of PMN function have been evaluated.

Glucocorticoids are extensively used therapeutically in cattle, but their effects on the immune system are not well characterized. Dexamethasone is a potent glucocorticoid which is commonly administered to cattle for the induction of parturition, the alleviation of physiologic udder edema, the reduction of musculoskeletal inflammation, and the treatment of ketosis (15). Dexamethasone administration to cattle has been observed to have serious detrimental consequences such as the recrudescence of infectious bovine rhinotracheitis virus (10, 11, 22), the predisposition to a fatal viremia in bovine viral diarrhea virus-infected calves (26), and a more severe disease course when administered to cattle suffering from bronchial pneumonia (5). The observed potentiating of infectious processes indicates that dexamethasone suppresses the bovine immune system. Dexamethasone has previously been shown to suppress mitogen-in
duced bovine lymphocyte blastogenesis (10, 20) which is considered an in vitro correlate of in vivo cell-mediated immunity.

The purpose of the experimentation reported here was to determine the effects of a pharmacological dose of dexamethasone on bovine PMN function. Six different functional parameters were evaluated on PMNs which were obtained from control and dexamethasone-treated cattle. All six tests were performed within a few hours of the time that the blood sample was drawn so that the results could be accurately compared.

**MATERIALS AND METHODS**

**Animals and dexamethasone treatment.** Apparently healthy adult Holstein-Friesian steers and bulls were used. Five animals served as controls, and seven animals were injected intramuscularly with 40 mg of dexamethasone (0.058 to 0.088 mg/kg of Azium; Schering Corp., Kenilworth, N.J.). Blood samples were obtained before and at 2 and 3, 7, 9, 10, and 13 days after dexamethasone administration for the evaluation of PMN function. The experimentation was conducted with groups consisting of two control animals and two or three dexamethasone-treated animals.

**PMN isolation.** PMNs were isolated as previously described (22a). Briefly, peripheral blood was collected into acid-citrate-dextrose solution and centrifuged, and the plasma and buffy coat layer were discarded. The packed erythrocytes were lysed with distilled water, and the remaining cells, which generally consisted of greater than 90% granulocytes, were washed and suspended in 0.015 M phosphate-buffered saline solution (pH 7.2) to a concentration of 5.0 x 10^6 PMNs (neutrophils plus eosinophils) per ml. The cells were held at room temperature and were used in all six PMN function tests within 3 h of the time they were standardized.

**PMN function tests.** The procedures for evaluating PMN function have been described in detail elsewhere (22a; Roth and Kaeberle, J. Immunol. Methods, in press). All PMN function tests were conducted in duplicate. Random migration under agarose was evaluated by measuring the area of PMN migration away from wells in the agarose after an overnight incubation at 37°C (22a). Heat-killed [1^25I]iododeoxuridine-labeled *Staphylococcus aureus* was used to evaluate ingestion by PMNs (22a). Opsonized [1^25I]-labeled *S. aureus* and PMNs were incubated together at 37°C with a bacterium/PMN ratio of 60:1. The extracellular *S. aureus* was removed by lysostaphin treatment, and the amount of PMN-associated radioactivity was determined. The results are expressed as the percentage of the [1^25I]-labeled *S. aureus* which was ingested. The quantitative Nitro Blue Tetrazolium (NBT) reduction assay was performed by adding 5.0 x 10^5 granulocytes to a suspension of opsonized zymosan in 1.0 ml of Earle balanced salt solution containing 0.4 mg of NBT (22a). After a 5.0-min incubation at 37°C the reaction was stopped, the purple formazan formed by the reduction of NBT was extracted with pyridine, and the optical density at 580 nm was determined. The results are expressed as optical density per 5.0 x 10^5 PMNs per 5 min in 5.0 ml of pyridine. Chemiluminescence was measured in a liquid scintillation spectrometer as previously described (22a), and the results are expressed as net counts per hour. Test vials contained 5.0 mg of preopsonized zymosan and 10^7 PMNs in a total volume of 10.0 ml of Gey's balanced salt solution. The standard reaction mixture for the determination of stimulated iodination contained 2.5 x 10^5 PMNs, 0.05 μCi of [1^25I]-labeled zymosan, and 0.5 mg of opsonized zymosan in 0.5 ml of Earle balanced salt solution (22a). The reaction was allowed to proceed for 20 min at 37°C, and then the amount of trichloroacetic acid-precipitable radioactivity was determined. The results are expressed as nanomoles of NaI per 10^5 PMNs per hour. The antibody-dependent, cell-mediated cytotoxicity (ADCC) assay was performed by utilizing 6^1Cr-labeled chicken erythrocytes as target cells (Roth and Kaeberle, J. Immunol. Methods, in press). The reaction mixture contained 2.5 x 10^5 Cr-labeled chicken erythrocytes and 2.5 x 10^6 PMNs (effector/target cell ratio of 10:1) in 0.5 ml of medium 199 containing 10% bovine anti-chicken erythrocyte serum. Triton X-100 controls, antibody controls, and PMN controls were included. After 3 h of incubation at 37°C, the reaction tubes were centrifuged, and a sample of supernatant solution was removed for gamma counting. The results are expressed as percentages of specific 6^1Cr release.

Statistical differences were calculated by using Student's *t* test and one degree of freedom per blood sample.

**RESULTS**

Dexamethasone treatment caused a marked increase in random migration by bovine PMNs (Fig. 1). The average area of random migration was slightly increased at 2 h postinjection and had doubled by 24 h postinjection. Random migration by PMNs had returned to within normal limits by 72 h after dexamethasone administration.

The ability of PMNs from dexamethasone-treated animals to ingest *S. aureus* was markedly depressed at 24 h postinjection (Fig. 2). By 48 h postinjection the ingestion ability had returned to within normal limits. At 10 days postinjection the ability of PMNs to ingest *S. aureus* was significantly enhanced.

Dexamethasone treatment markedly suppressed NBT reduction by PMNs (Fig. 3). The suppression was apparent by 2 h postinjection and remained for several days. Chemiluminescence, another parameter of the oxidative metabolism of the PMN, showed a similar pattern of suppression after dexamethasone treatment (Fig. 4).

Iodination by PMNs isolated from dexamethasone-treated cattle was somewhat depressed by 2 h postinjection (Fig. 5). By 24 h postinjection, the iodination value was less than 50% of normal. The iodination value remained at approximately
50% of normal for the entire 13-day period after a single injection of dexamethasone.

ADCC by PMNs was somewhat depressed at 2 h and was markedly depressed at 24 h postinjection (Fig. 6). By 48 h postinjection it had returned to near normal levels.

The percentage of eosinophils in the PMN preparations was markedly reduced after dexamethasone administration and remained low for the entire 13-day period (Fig. 7).

DISCUSSION

A single pharmacological dose of dexamethasone administered to normal cattle had a profound effect on PMN function. Dexamethasone administration caused an enhancement of random migration (chemokinesis) by the PMNs but a depression of ingestion, oxidative metabolism, the myeloperoxidase—H₂O₂—halide antibacterial system, and ADCC by PMNs.
The stimulation of random migration observed after dexamethasone administration is similar to results reported for PMNs from human patients being treated with prednisolone for various pathological conditions (27–29). Stevenson et al. demonstrated that the enhancement of migration was indirect and due to a mononuclear phagocyte-produced peptide factor which stimulated PMN migration (27–29). It is not known whether the increase in PMN random migration which we observed was mediated through an effect of dexamethasone on mononuclear leukocytes or whether the dexamethasone had a direct stimulatory effect on PMN migration.

There are conflicting reports on the effect of
corticosteroids on ingestion by human PMNs. Corticosteroids have been reported to inhibit ingestion by PMNs (8, 13) and to have no effect on ingestion (3, 17). These discrepancies may be due to differing techniques for evaluating ingestion or to differing amounts and types of corticosteroids used. None of these reports involved the in vivo administration of a pharmacological dose of corticosteroid to a normal individual. Our results indicate that a single pharmacological dose of dexamethasone administered in vivo will inhibit ingestion by bovine PMNs. The S. aureus assay performed here evaluates the initial rate of ingestion rather than the capacity for

Fig. 5. Effect of in vivo dexamethasone administration on iodination by bovine PMNs. Values and statistically significant differences are as in Fig. 1, except that n = 50 for the control group.

Fig. 6. Effect on in vivo dexamethasone administration on ADCC by bovine PMNs. Values and statistically significant differences are as in Fig. 1, except that n = 39 for the control group, and there were five animals in the dexamethasone group.
ingestion (22a). There is no apparent explanation for the observed increase in S. aureus ingestion 10 days after dexamethasone administration. This increase in PMN function was noted on only 1 day in only one of the PMN function tests and was of only marginal statistical significance. We feel that the data are not sufficient to attach any importance to this observation.

Our results also demonstrate that dexamethasone administration inhibits NBT reduction and chemiluminescence, two parameters of PMN oxidative metabolism. Inhibition of NBT reduction has been reported previously after in vitro treatment of human PMNs with hydrocortisone (3, 17) and in PMNs from patients with pathological processes who were being treated with corticosteroids (3, 4). The depression of NBT reduction and chemiluminescence indicated that an important component of the PMNs bactericidal mechanism is impaired by dexamethasone administration (1, 2).

Dexamethasone administration caused a dramatic, prolonged depression of iodination by the bovine PMN preparations. The in vitro treatment of human PMNs with high dosages of dexamethasone has also been reported to inhibit iodination (8). The iodination reaction is a measure of the activity of the myeloperoxidase-hydrogen peroxide-halide antibacterial system in the PMN and it may be depressed by an inhibition of PMN ingestion, oxidative metabolism, or degranulation (14). Our results indicate that PMN ingestion and oxidative metabolism are inhibited by dexamethasone. However, the prolonged suppression of iodination (Fig. 5) as compared with S. aureus ingestion (Fig. 2), NBT reduction (Fig. 3), and chemiluminescence (Fig. 4) suggests that another mechanism is partially responsible. The PMN preparations used in this study contained a mixed population of eosinophils and neutrophils. Eosinophils in the peripheral blood of normal cattle may make up from 2% to 20% of the total leukocyte population (25); this results in a fairly high percentage of eosinophils in the purified PMN preparations from normal animals (an average of 22.7% in this study). Corticosteroid administration causes eosinophils to sequester in lymphoid tissues (24) and thereby reduces the number of circulating eosinophils in the peripheral blood. This results in PMN preparations from dexamethasone-treated animals with a very low percentage of eosinophils (Fig. 7). The depression of iodination (Fig. 5) seems to parallel the depletion of eosinophils in the PMN preparation (Fig. 7). We have shown that bovine eosinophils are four to five times more active than bovine neutrophils in the stimulated iodination reaction (Roth and Kaaberle, J. Immunol. Methods, in press). Therefore, the depression of iodination is probably mainly due to the lowered percentage of eosinophils in the PMN preparation. We believe that the early depression of iodination was partially due to a direct effect of dexamethasone on the
iodination ability of the neutrophils and not totally due to a reduction in eosinophil numbers. In other experimentation we have demonstrated that administration of adrenocorticotropic hormone to cattle with very low numbers of eosinophils in their peripheral blood resulted in a depression of iodination by PMNs without a corresponding decrease in eosinophil numbers (J. A. Roth, M. L. Kaebler, and W. H. Hsu, Am. J. Vet. Res., in press).

The inhibition of ingestion, oxidative metabolism, and ADCC by PMNs after dexamethasone administration is probably not due to the decreased percentage of eosinophils because: (i) we have shown that eosinophils are less efficient than neutrophils at ingestion of S. aureus and ADCC, and the two cell types produce equivalent NBT reduction (Roth and Kaebler, J. Immunol. Methods, in press), and (ii) these parameters returned to normal within a few days, whereas iodination and the percentage of eosinophils remained depressed for the entire period of the study.

Bovine neutrophils have been demonstrated to be the most active bovine cell type tested in mediating ADCC against herpesvirus-infected target cells (23). The exact mechanism of this cytotoxicity is not known, but it is not dependent upon deoxyribonucleic acid, ribonucleic acid, or protein synthesis within the PMN (7, 30), and seems to require direct cell membrane contact (12). The results observed here of depressed ADCC mediated by PMNs after the in vivo administration of dexamethasone support the previous report of depressed ADCC by bovine PMNs treated in vitro with dexamethasone (30). This inhibition of the ability of PMNs to mediate ADCC may play an important role in allowing bovine herpesvirus, which has been recrudesced by the administration of dexamethasone, to multiply (10, 11, 22).

In summary, a single pharmacological dose of dexamethasone administered to cattle caused an enhancement of random migration by PMNs but an impairment of (i) the ingestion of bacteria by PMNs, (ii) the oxidative metabolism of the PMN, (iii) the myeloperoxidase-hydrogen peroxide-halide antibacterial system of the PMN, and (iv) antibody-dependent, PMN-mediated cytotoxicity. The impairment of PMN function may at least partially explain the detrimental effects associated with dexamethasone administration to cattle (5, 11, 26).

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LITERATURE CITED


