Excretion of $[^3]H\text{prednisolone}$ in clinically normal and experimentally infected bovine udders

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The excretion rate of $[^3]H\text{prednisolone}$ from clinically normal and experimentally infected udders of 10 lactating cows was studied. Each quarter of 6 cows was injected with a single dose of $[^3]H\text{prednisolone}$ mixed with nonradioactive prednisolone equivalent to 10 mg in 10 ml of peanut oil base. Each of the remaining 4 cows was given 40 mg of nonradioactive prednisolone and $[^3]H\text{prednisolone}$ radioactive prednisolone equivalent to 10 mg in 10 ml of peanut oil base. Control and postadministration samples of blood, milk, and urine were examined for radioactivity. The effects of $[^3]H\text{prednisolone}$ were evaluated in the same cows, first in clinically normal udders, then 2 weeks later in udders experimentally infected with Streptococcus agalactiae.

Absorption and elimination of prednisolone were the same before and after induced infection. Within 3 hours after intramammary injection, 95% of the labeled prednisolone was absorbed systemically, <5% of this dose was recovered in milk, and 29% was excreted in urine. After IV injection of $[^3]H\text{prednisolone}$, <0.2% of the total radioactivity was recovered in milk and <46% was excreted in urine.

Clinical mastitis induced by S agalactiae was moderate. Circulating blood leukocytes and somatic cells in the milk of normal cows remained essentially unchanged. The leukocyte response to induced infection was rapid in blood and milk. Large numbers of leukocytes were noticed in the milk and a severe leukopenia occurred. Prednisolone treatment did not alter the number of somatic cells in milk or reduce the inflammatory response of experimentally infected cows.

Corticosteroids have been used for many years as a supplement to standard antibiotic therapy in the treatment of bovine mastitis. In general, 2 rationales support the incorporation of steroids in intramammary preparations: (i) prevention or diminution of the clinical signs of inflammation induced by a mildly irritating product or vehicle and (ii) reduction of the inflammatory response and potential tissue damage in acute clinical mastitis. Extensive use of corticosteroids in the treatment of mastitis have attracted only a few reports of effects in local intramammary therapy.1

Prednisone and prednisone acetate produced marked anti-inflammatory effects, a rapid reduction in leukocyte counts in affected quarters, and a faster return to normal when used alone or in conjunction with antibiotics.1,2 In contrast, prednisone neither hastened recovery nor affected leukocyte counts when an antibiotic ointment with or without 4.5 mg of prednisone acetate was injected into 62 mastitic quarters.3

Swarbrick4 could not show an advantage when 10 mg of betamethasone, 50 mg of prednisone, or 50 mg of prednisone trimethyl acetate was added to an aqueous preparation of oxytetracycline. Administration of cortisol:corticosterone at a ratio of 8:1 for several consecutive days had little effect on the concentration of leukocytes in milk.5 In a study6 using Aerobacter aerogenes to induce mastitis, large doses of 9a-fluoroprednisolone acetate injected into mammary glands or given IM failed to inhibit or delay infiltration of leukocytes into mammary quarters exposed to coliform organisms. 9a-Fluoroprednisolone acetate (5 or 10 mg) markedly altered numbers of blood leukocytes, but had little effect on the number of somatic cells in milk.7

Investigations in cattle and dogs indicated that most corticosteroids are excreted in the urine and feces. Krzeminski et al8 injected methylprednisolone into udders of dairy cows but were unable to detect this compound in milk 24 hours after administration. In dogs, approximately half of the $[^3]H\text{6a-methylprednisolone-21}-\text{acetate given IM}$ was excreted in feces, and about a quarter of the dose was present in urine after 42 days.9

Little is known about the relative value and the mechanism of corticosteroid activity in the bovine udder. The effectiveness of local corticoid therapy in suppressing the inflammatory response in the udder has been questioned. The purpose of the present study was to define the anti-inflammatory effects of prednisolone on the clinically normal and the mastitic bovine udder during lactation and to examine systemic absorption of the drug and its rate of excretion in milk and urine after single intramammary or IV administration.

**Materials and Methods**

$[^3]H\text{6,7-Prednisolone}$ lots with specific activities of 53 and 58 Ci/mmol were used. Authenticity of the labeled prednisolone was determined by methods involving thin-layer chromatography and radiochemical assays. Authenticity of the labeled prednisolone was confirmed by the Amersham Corp, Arlington Heights, Ill.
was established by thin-layer chromatography on silica gel by the chloroform:ethanol (80:20) system. The radiolabeled and nonradioabeled prednisolone was cochromatographed and the radioactive prednisolone was > 99% pure.

Castle and experimental design—Ten lactating Holstein cows weighing 500 to 700 kg were housed, fed, and milked under normal conditions. Prednisolone was investigated first in clinically normal udders (normal phase) and then after 2 weeks in experimentally infected udders of the same cows (infected phase).

A single dose of [3H]prednisolone was injected into each quarter of the mammary glands of 6 healthy cows; 4 other healthy cows were given a single IV dose of [3H]prednisolone. Two weeks after the normal phase, mastitis was induced in all cows by infection with Streptococcus agalactiae, and another single dose of [3H]prednisolone was administered as soon as clinical infection in the udders was established. The [3H]prednisolone was used to monitor the absorption and elimination of prednisolone in normal and infected bovine udders.

Intramammary injections were given immediately after the morning milking was completed. Each quarter was injected with a single dose of [3H]prednisolone (13.5 to 33.8 µCi) and with nonradioactive prednisolone equivalent to 10 mg of prednisolone in 10 ml of peanut oil (40 mg/cow). All cows were milked with a commercial milker, and composite milk samples were collected at 0.0, 1.5, 3, and 6 hours after injection on the 1st day and twice daily (9:00 AM and 4:00 PM) thereafter for 3 days. Milk yield was measured by weight daily, and aliquots (approx 100 ml) of milk were examined for radioactive content.

Urine was collected via indwelling catheters. Total urine weight was recorded at each milking (9:00 AM and 4:00 PM) and aliquots (approx 100 ml) of urine were taken for measurement of radioactivity. Blood was drawn from a jugular vein at 0 and 0.5 hours, and hourly thereafter for 7 hours on the 1st day and at each milking for 4 days. Blood was centrifuged and aliquots from the harvested serum were taken immediately for analysis. The remainder of the serum was frozen. Heparinized blood samples were collected at each milking for total leukocyte and differential counts.

A single dose of [3H]prednisolone, mixed with nonradioactive prednisolone, equivalent to 40 mg in 3.5 ml of 60% ethanol was injected into the jugular vein of 4 clinically normal lactating cows. Two weeks after the normal phase, the udders of 3 cows (1 cow in the late stage of lactation was released from study) were experimentally infected as described earlier, and the cows were again given a single IV dose of [3H]prednisolone. Milk, urine, and blood were collected as described previously with additional blood samples collected 15 and 45 minutes after injection. Blood was drawn from the jugular vein contralateral to the side of injection.

Dose preparation and injections—The dose for intramammary infusion was prepared from labeled and unlabeled portions of prednisolone dissolved in 95% ethanol. Dissolved [3H]prednisolone (13.5 to 33.8 µCi) was combined with nonradioactive prednisolone and peanut oil to obtain a mixture containing 10 mg of prednisolone in 10 ml of peanut oil. Ethanol was evaporated from the oil under vacuum (138 kPa) for 24 hours. Total radioactivity was determined in aliquots taken from each injection dose, and each dose was then transferred to a disposable syringe for intramammary instillation. Radioactivity loss was determined by using ethanol to extract residual oil from used syringes and vials.

Solution for IV injection was prepared by mixing dissolved [3H]prednisolone (100 to 164 µCi) and nonradioactive prednisolone equivalent to 40 mg in 3.5 ml of 60% ethanol. Each dose containing 40 mg of prednisolone was transferred to a 5-ml syringe. Radioactivity lost after injection was determined as previously described.

Radioactive determination—Radioactivity was measured in a liquid scintillation system. The counting efficiency for [3H] was 46%. Aliquots of 500 µl (each) of milk, serum, and urine were placed directly in scintillation counting vials, and 10 ml of scintillation fluid was added. The contents were mixed and counted in a conventional manner. [3H]Prednisolone was used as the internal standard, and samples were corrected for variations in counting efficiencies (background and color quenching). Results were expressed as disintegrations per minute (dpm). Counting efficiencies for radioactivity in urine, serum, and milk were 62%, 63%, and 75%, respectively.

Inoculum—A culture of S. agalactiae, Cornell 84 strain, was used to induce mastitis. An overnight broth culture of S. agalactiae was swabbed onto the surface of large soybean-casein agar plates and was incubated at 37 °C for 18 hours. Cells were washed with sterile 0.85% saline solution, centrifuged, and suspended with saline solution or Trypticase soy broth. Inoculum containing 1.0 to 1.5 ml of the S. agalactiae culture was used to induce infection. Each quarter was injected with a single inoculum immediately after the morning milking. At the subsequent evening milking, all exposed udders were partially milked; thereafter, normal milking procedure was followed. The exposed cows were observed and the mammary glands were examined for local effects.

Before each milking, foremilk was examined for physical characteristics by using the strip-up technique. Fresh foremilk samples from each quarter (6 and 24 hours after inoculation) were collected aseptically, plated, and examined for the presence of mastitis-inducing pathogens. When examination of inoculated quarters exhibited clinical signs of mastitis, intramammary or IV administration of [3H]prednisolone was generally initiated. A single dose of the [3H]prednisolone was generally administered 24 hours after inoculation of the udder. The minimal criteria for udder infection were colonization and recovery of S. agalactiae from foremilk samples, marked leukocytic exudation into the milk, abnormal secretion, and obvious clinical signs of mastitis associated with edema and inflammation.

Bacteriologic procedures and leukocyte and somatic cell determinations—Foremilk samples taken from individual quarters were examined microbiologically. Microorganisms were recovered and identified according to bacteriologic procedures previously described. Blood leukocytes were counted electronically in a Coulter counter, and blood smears were stained with Wright's stain for differential leukocyte determinations. Somatic cells in milk were counted by the standard direct microscopic somatic cell count method. Milk smears were prepared, air dried, and stained with Levoritz-Weber or pyronin Y-methyl green stains.

Results

Intramammary injection—Mean values of radioactivity recovered in urine collected from infected and normal cows accounted for 25.4 ± 6.4% and 29.6 ± 10.5%, respectively, of the total dose administered (Table 1). Most of the radioactivity in the urine was excreted within 30 hours. Recovery of radioactivity in milk collected from infected udders and normal udders within 24 hours after prednisolone injection was 2.0 ± 0.9% and 5.0 ± 2.7%, respectively. Thereafter, radioactivity in milk was negligible.

1 Sigma Chemical Co, St Louis, Mo.
2 Bardex Foley catheter, 20 French size, 75-ml balloon, C R Bard Inc, Murray Hill, N.J.
Radioactivity in serum was detected 30 minutes after intramammary injection; prednisolone radioactivity peaked within 2 hours of injection and gradually declined by 9 hours after administration. Activity values as disintegrations per minute per milliliter were averaged from 6 lactating cows in the normal and infected phases (Fig 1).

Milk production and urinary excretion were determined for each cow over 4 days (Table 1). Total urinary output from cows in the normal and infected phase was similar (77.2 ± 23.1 kg and 76.3 ± 25.7 kg, respectively). Radioactivity in urine decreased to marginally detectable amounts by 78 hours after administration. Average total milk yield was 60.27% (P > 0.01) lower during the infected phase than during the normal phase (44.5 ± 20.0 kg and 112.0 ± 33.1 kg, respectively).

IV phase—Radioactivity recovered in urine from normal and infected phases accounted for 46.0% and 42.6% of the total dose, respectively (Table 2). Low radioactivity levels were detected in milk, with recovery averaging from 0.05% to 0.13% of the total dose administered. As expected, radioactivity was detected in both phases 15 minutes after IV administration of [3H]prednisolone, but was not detectable after 48 hours. Radioactivity as disintegrations per minute per milliliter in serum was plotted from the data obtained during the normal and infected phases (Fig 2).

Radioactivity in milk and urine was detectable in both phases at the 1st milking (6 hours) but diminished to trace levels by 54 hours and was eliminated in urine by 78 hours. The accumulated urine production during the 4 days from normal and infected phases was 43.4 ± 10.7 kg and 47.7 ± 16.7 kg, respectively (Table 2). Milk production during the infected phase was 53.06% (P > 0.025) lower than that of the normal phase (22.2 ± 10.7 kg and 47.3 ± 6.2 kg, respectively).

Clinical observations—Overall physical condition of cows used in the normal phase of study was good and feed...
consumption was normal. Signs of gross abnormalities or changes in the physical character or quality of milk were not noticed. All quarters were functional and appeared to be free of clinical mastitis.

Gross clinical reactions of the cows were moderate after exposure to *S. agalactiae*. Twelve to 24 hours after inoculation, a marked inflammatory response associated with a massive leukocytosis in the milk was noticed. Clinical signs in affected cows were edema of the udder with swollen, firm, and tender quarters; watery, flaked, or thickly clotted milk; and a marked decrease in milk production. *Streptococcus agalactiae* was isolated from fresh postexposure milk samples and was identified by standard procedures. In most cows, the swelling of quarters reached the maximum within 24 to 36 hours and subsided in subsequent milkings; milk generally returned to normal appearance after 4 days.

**Blood leukocytes and somatic cell response**—In normal, noninfected cows, the effect of prednisolone on blood leukocytes after intramammary or iv administration was similar for both groups of cows (Fig 3 and 4). A small transitory peak in blood leukocytes after prednisolone administration was associated with changes in differential leukocyte profiles and a progressive increase in neutrophils. Regardless of the dose or route of administration, the response to prednisolone was essentially the same. The maximum increase in circulating leukocytes of > 3,000 leukocytes/mm³ of blood occurred 7 hours after administration, decreased within 12 hours, then returned to baseline values (Fig 3 and 4).

In milk, somatic cell counts fluctuated within the established normal range for these cows and revealed no changes in concentration after prednisolone administration (Fig 3). The concentration of somatic cells in 2 of 4 cows given iv injections remained at normal values during the study, but indicated a considerable increase in cell count values in 2 other cows (Fig 4). This increase was attributed to a transient inflammation caused by the presence of hemolytic staphylococci detected in 3 quarters after the start of the trial. The affected quarters of the spontaneously infected cows showed neither clinical signs of mastitis nor gross changes in secretion.

**The leukocyte response to the infection** (Fig 5 and 6) was rapid in blood and milk. A fast outpouring of leukocytes into the milk of infected quarters was noticed. Overall udder reactions 24 hours after exposure were pronounced, and the somatic cell counts in milk (16 to 24 million cells/ml) were comparable for both groups of cows. This high concentration of somatic cells in milk remained
essentially unchanged after prednisolone treatment and persisted at somewhat decreased amounts in most cows. A severe leukopenia occurred within 6 hours after infection, with an apparent massive exudation of leukocytes into the milk of the infected glands. As a consequence, there was a precipitous decrease in the total leukocyte count of blood (from 8,000 to 3,000 leukocytes/mm³), followed by a return toward baseline. Examination of clinical and analytical data indicated that except for a small transitory increase in circulating blood leukocytes, prednisolone treatments at the doses used neither affected the concentration of somatic cells in milk nor altered the degree of inflammation in glands of infected cows. Even though all quarters of cows were treated at once, the response obtained was not sufficient to cause decrease in swelling and overall clinical improvement of infected glands.

Discussion

Absorption of prednisolone (based on radioactivity) from clinically normal and infected udders was rapid, peaking in serum at 2 hours after injection. Radioactivity levels in milk were consistently below those in serum, and radioactivity was not detected in milk by 3 hours. Most of the radioactivity absorbed from the udder into the systemic circulation was excreted in the urine and presumably in the feces. Values of radioactivity recovered from urine of infected and control cows accounted for 25.4% and 29.6%, respectively.

In human subjects, 90% of the radioactivity administered as [14C]prednisolone was excreted in urine within 48 hours and < 2% was excreted in feces. After oral administration of [3H]6alpha-methylprednisolone, 21-acetate in the dog, 25% to 31% of the radioactivity was recovered in urine and 44% to 52% in feces. In the ruminant, the major route for prednisolone excretion is presumably via the feces. The accumulated percentages in urine from an ovariectomized cow after iv administration of [14C]corticosterone and [14C]cortisol were 20.2% and 29.0%, respectively. In our experiments, the recovery of radioactivity in urine after iv injection ranged from 42% to 46%.

The radioactivity content in milk after intramammary injection (< 5% of the total dose) and iv injection (< 0.13% of the total dose) and clinical response observed in this study indicate that the concentrations of prednisolone produced were generally below the clinically effective concentration. Evidently, prednisolone is quickly absorbed from the udder into the systemic circulation and is quickly eliminated. After iv injection, prednisolone is also rapidly eliminated, and little is present in milk.
The induction of clinical mastitis by experimental infection of the udder with *S. agalactiae* did not appear to affect the absorption and excretion of \[^3H\]prednisolone in milk and urine after intramammary or IV injection. Compared with the normal phase, milk production of cows in the infected phase decreased by a factor of one-half to one-third. The decrease in milk production could have resulted from the active mastitis in the mammary gland.

Circulating blood leukocyte response to the induced infection was similar to that reported previously. The lack of a somatic cell change in milk after prednisolone treatment is consistent with earlier reports and results of therapy with cortisol:corticosterone, prednisone, and 9α-fluoroprednisolone. In the present study, the local instillation of prednisolone into the udder at the dosage rates used indicated no advantage. Because prednisolone does not remain in the udder long, the anti-inflammatory activity of this compound at the level of epithelium of the udder is inadequate to inhibit or change existing inflammation in infected quarters.

In acute cases of mastitis, the corticosteroids, when given systemically in large doses, appear to enhance the overall clinical response, presumably by their anti-inflammatory and antitoxic actions. Data from the present study indicated no change in the acute signs of inflammation and did not contribute to the determination of the mechanism of corticosteroid activity in the udder.

### References