ABSORPTION, EXCRETION AND TISSUE RESIDUE IN FEEDLOT HEIFERS INJECTED WITH THE SYNTHETIC PROSTAGLANDIN, FENPROSTALENE

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Summary

Preliminary studies on use of the synthetic prostaglandin, fenprostalene, as an abortifacient had indicated that maximum effectiveness was dependent upon slow delivery. Because both route of administration and formulation control delivery rates, the influences of intramuscular (im) vs subcutaneous (sc) injections, and aqueous acetate buffer (AAB) vs polyethylene glycol-400 (PEG) vehicles on the plasma concentration and urinary excretion of fenprostalene were compared. Feedlot heifers were administered 1 mg injections of [13,14-3H] -fenprostalene. Blood samples and total urine excretion were collected during the following 96 h. The maximum concentration of tritium in plasma occurred at 2 h for AAB-im (.90 ng eq/ml), PEG-im (.75 ng eq/ml) and AAB-sc (.64 ng eq/ml), and then declined throughout 24 h with t½ values of 6.1, 9.4 and 9.2 h, respectively. The peak concentration from PEG-sc was lower (.37 ng eq/ml, P<.05), observed later (4 h, P<.05) and declined more slowly following peak concentration (t½ = 15.1 h, P<.05). Consistent with delayed absorption, a smaller fraction (P<.05) of the total radioactivity excreted in urine was recovered during the first 24 h after injection for PEG-sc (85%) than for PEG-im (95%), AAB-sc (97%) or AAB-im (99%). In a tissue distribution study, plasma, urine and fecal samples were collected and heifers were slaughtered at various times following sc injection of 1 mg of [3H] fenprostalene in PEG. Peak concentrations of tritium in plasma occurred between 4 and 8 h and declined with a t½ of 15.2 h. Urine contained 60% and feces 40% of total recovered radioactivity. Drug equivalent concentrations in edible tissues 24 h after injection were .74, 2.25, .09 and .15 ppb in liver, kidney, muscle and fat, respectively, and continued to decline. Formulation of fenprostalene in PEG for sc injection resulted in a sustained-release prostaglandin. Nonetheless, the compound was rapidly excreted in urine and feces, leaving no persistent tissue residue.

(Key Words: Fenprostalene, Absorption, Excretion, Tissue Residue.)

Introduction

Fenprostalene3, a synthetic prostaglandin analog that differs from prostaglandin F2α (PGF2α) by modifications of both side chains (figure 1), recently was accepted as safe and effective as an abortifacient in feedlot heifers when injected sc in a 1 mg dose (FDA, 1983). The molecule was designed with chemical substitutions that resist metabolism and, consequently, should have a predictably extended t½ in plasma compared with PGF2α (Muchowski and Fried, 1976). Nonetheless, early studies with fenprostalene suggested that its efficacy as an abortifacient in heifers was enhanced when 1 mg of an aqueous formulation was infused continuously over 24 h compared with a single, im injection of an equivalent dose (R. C. Herschler, unpublished data). Because formulation and route of injection would affect absorption rate, the influences of formulating fenprostalene in either an aqueous buffer or polyethylene glycol-400, and injecting those solutions either im or sc were studied. Subsequently the patterns of absorption, excretion and depletion of tissue residues were monitored following injection of the most slowly absorbed dosage form.

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Materials and Methods

Fenprostalene was tritiated at carbon atoms 13 and 14 (figure 1). Radiochemical purity was 96% as determined by a radiochromatographic scan after TLC chromatography on silica gel 60F-254 using ethyl acetate:methanol (80:20 v/v). Before dosing, animals were placed in individual metabolism crates and were fitted with indwelling urinary catheters to enable separate collection of urine and feces. A diet consisting of 60% concentrate and 40% alfalfa hay was fed throughout both trials.

Six heifers averaging 210 kg of body weight (SE = 3.2 kg) were used in trial 1 to study absorption of \(^{3}\text{H}\)fenprostalene delivered in different vehicles and by different routes of injection. Tritiated fenprostalene was prepared in two injectable formulations at a specific activity of 3mCi/mg. One solution contained .5 mg of \(^{3}\text{H}\) fenprostalene/ml in aqueous, 250 mM sodium acetate buffer, pH 5; the other preparation contained .5 mg of \(^{3}\text{H}\)fenprostalene/ml in polyethylene glycol-400 (PEG). Animals in this study were injected with 1 mg of fenprostalene either im or sc in the lateral area of the neck. Each animal received two injections with a 1-wk interval between injections to allow clearance of tritium from the previous dose.

At scheduled collection times, 10 ml samples of blood were drawn into heparinized tubes by jugular venipuncture and plasma was separated by centrifugation at 8,000 × g. Total urine output was measured and 25-ml samples were saved at each collection. All samples were held at 4 C until they were analyzed. Duplicate aliquots of plasma, .2 to .6 ml, and urine, .005 to .6 ml, were assayed by combining with 10 ml of Oxifluor scintillation fluid followed by counting in a Packard Model 3330 scintillation counter. Counting efficiencies were determined using \(^{3}\text{H}\)toluene as an internal standard. Statistical analysis was by analysis of variance (SAS, 1979) and comparisons of individual treatment means were made by LSD when treatment effects were indicated (P<.05).

For the tissue distribution study (trial 2), fenprostalene was formulated according to specifications of the marketed product and contained .5 mg of fenprostalene and .5 mg of dl-α-tocopherol/ml in PEG. Ten heifers averaging 317 kg (SE = 7 kg) received 1 mg of \(^{3}\text{H}\)fenprostalene by the sc route in the lateral area of the neck. Animals slaughtered within 7 d following dosing were injected with \(^{3}\text{H}\)fenprostalene having a specific activity of 4.013 mCi/mg, whereas animals to be slaughtered after a period longer than 7 d after dosing were injected with material containing 12.122 mCi/mg.

Plasma and urine were collected for 7 d, stored and analyzed as described for trial 1. Feces obtained from each animal at each collection were blended for 10 min in a Hobart mixer, and 10 g samples were stored at 4 C prior to analysis. Two heifers/day were slaughtered at 1, 7, 14 and 28 d and one heifer/d was slaughtered at 3 and 21 d after injection. One kilogram samples of liver, perirenal fat and muscle from the bottom round were saved at slaughter. Both kidneys were collected and a 25 cm diameter, 5 cm deep section of tissues at the injection site was excised. Tissue samples were minced by repeated passes through a meat grinder and were stored frozen before analysis. Triplicate samples, 40 to 300 mg, of feces and tissues were weighed, dried at 60 C and combusted in a Packard Sample Oxidizer, Model 306. The \(^{3}\text{H}\)O formed was flushed with \text{H}_2\text{O}...
into 15 ml of scintillation fluid and counted. Efficiency in recovering radioactivity from the procedure was determined by known additions of \([^3\text{H}]\) fenprostalene to samples before combustion. Counting efficiencies were determined using \([^3\text{H}]\) toluene as an internal standard.

**Results**

Maximum tritium concentration in plasma after either im or sc injection of \([^3\text{H}]\) fenprostalene in acetate buffer in trial 1 was observed in the first sample (2 h) obtained after injection (table 1). The radiochemical equivalent concentrations of fenprostalene were .90 and .64 ng eq/ml for the im and sc routes of injection, respectively, and declined through 24 h with \(t_{1/2}\) values of 6.1 and 9.2 h, respectively. Intramuscular injection of the PEG formulation resulted in a maximum concentration (.75 ng eq/ml), time of maximum concentration (2 h) and \(t_{1/2}\) (9.4 h) similar to sc injection of the acetate buffer formulation. However, when the PEG formulation was injected sc, maximum concentration of fenprostalene (.37 ng eq/ml) was lower (\(P<.05\)) and was observed later (4 h; \(P<.05\)) compared with the other combinations of vehicle and injection route. Similarly, the \(t_{1/2}\) of tritium in plasma was extended to 15.1 h reflecting a slower decline in the plasma concentration after sc injection of the PEG formulation (\(P<.05\)).

Total recovery of injected tritium in urine throughout 72 h ranged from an average of 80.8% for im injection of the acetate buffer formulation to 65.3% for sc injection of the PEG formulation (table 1). Apparent differences were not statistically detectable (\(P>0.05\)). However, heifers injected sc with the PEG formulation excreted less radioactivity in urine (85.1%; \(P<0.05\)) during the first 24 h after injection. Consequently, variables measured in both plasma and urine indicated that the rate of absorption of fenprostalene was substantially slowed when the material was formulated in PEG and injected sc.

Subcutaneous injection with 1 mg of \([^3\text{H}]\) fenprostalene in PEG for the tissue distribution study (trial 2) resulted in a plasma specific activity profile nearly identical to the sc injection of the PEG formulation observed in trial 1 (figure 2). The maximum concentration of fenprostalene (.21 ng eq/ml) was observed at 8 h after injection and then declined through the first 24 h with a \(t_{1/2}\) of 15.2 h. Beyond 48 h the average concentration of tritium in plasma equated to less than .06 ng eq/ml of fenprostalene, and continued to decline thereafter with a \(t_{1/2}\) of approximately 5 d. Recovery of the total dose in urine averaged 48% and was essentially

<table>
<thead>
<tr>
<th>Sample</th>
<th>Formulation and route of injection</th>
<th>Acetate buffer</th>
<th>Polyethylene glycol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intramuscular</td>
<td>Subcutaneous</td>
<td>Intramuscular</td>
</tr>
<tr>
<td></td>
<td>Maximum fenprostalene concentration, ng eq/ml</td>
<td>.90(^a)</td>
<td>.64(^a)</td>
<td>.75(^a)</td>
</tr>
<tr>
<td></td>
<td>Time of maximum concentration, h</td>
<td>2(^a)</td>
<td>2(^a)</td>
<td>2(^a)</td>
</tr>
<tr>
<td></td>
<td>Half-life(^d), h</td>
<td>6.1(^a)</td>
<td>9.2(^b)</td>
<td>9.4(^b)</td>
</tr>
<tr>
<td>Urine</td>
<td>Total recovery in 72 h, %</td>
<td>80.8</td>
<td>72.6</td>
<td>70.9</td>
</tr>
<tr>
<td></td>
<td>Fraction recovered by 24 h, %</td>
<td>98.7(^a)</td>
<td>96.9(^a)</td>
<td>94.6(^a)</td>
</tr>
</tbody>
</table>

\(^{a,b,c}\)Means in the same row with different superscripts differ (\(P<.05\)). Values are means of three injections.

\(^{d}\)Fractional disappearance rates were calculated by least-squares linear regression of plasma concentrations between maximum concentration and 24 h after injection using the equation \(\ln C = \ln C_0 - kt\), where \(C =\) concentration, \(t =\) time in h, and \(k =\) fractional disappearance rate. Half life was calculated as \(t_{1/2} = \ln 2/k\). Statistical analysis was performed on the \(k\) values.
tamin in urine and feces (table 2). Concentrations of tritium in tissues taken 24 h after injection were low, but were severalfold higher in kidney (2.25 ppb) and liver (.74 ppb) than in muscle (.09 ppb) or fat (.15 ppb). Based on observed excretion rates, the higher concentrations in kidney and liver apparently reflected the role of those two organs in excretion of fenprostalene in urine and feces, respectively.

To resolve and evaluate potential metabolites of fenprostalene in trial 2, aliquots of urine from each collection time were composited by volume to prepare a representative sample of urine excreted throughout the first 24 h after injection. Subsamples of that composite sample were adjusted to pH 2, pH 6 and pH 8, and were extracted with three volumes of ethyl acetate. The percentage of total urine radioactivity extracted was 92, 23 and 3% for samples adjusted to pH 2, pH 6 and pH 8, respectively, suggesting that at least 97% of the injected fenprostalene had been converted to acidic metabolites. Upon TLC separation of the ethyl acetate fraction on silica gel-G developed in ethyl acetate:methanol:acetic acid (80:19:1 v/v), only one component identified by TLC and mass spectroscopy as the free carboxylic acid form of fenprostalene was found. Consequently, these results indicate that fenprostalene was rapidly deesterified, but that the free acid resisted further metabolism. Results from subsequent in vitro studies have substantiated that hydrolysis of the methyl ester of fenprostalene to the stable free acid occurred with a t½ of 15 min when 150 pg of [3H]fenprostalene was incubated with 1 ml of bovine plasma (G.J.-L. Lee and S. Kushinsky, unpublished data).

### Table 2. Cumulative Excretion of Tritium in Urine and Feces (Trial 2)

<table>
<thead>
<tr>
<th>Time of collection, h</th>
<th>No. of heifers</th>
<th>Percentage of dose ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urinary excretion</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>6.8 ± .8</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>15.7 ± 1.5</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>24.4 ± 2.1</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>40.9 ± 3.3</td>
</tr>
<tr>
<td>48</td>
<td>8</td>
<td>48.5 ± 4.9</td>
</tr>
<tr>
<td>72</td>
<td>8</td>
<td>49.2 ± 4.9</td>
</tr>
<tr>
<td>96</td>
<td>7</td>
<td>47.8 ± 5.3</td>
</tr>
<tr>
<td>120</td>
<td>7</td>
<td>47.9 ± 5.3</td>
</tr>
<tr>
<td>144</td>
<td>7</td>
<td>48.0 ± 5.3</td>
</tr>
<tr>
<td>168</td>
<td>7</td>
<td>48.0 ± 5.3</td>
</tr>
</tbody>
</table>
TABLE 3. CONCENTRATION OF FENPROSTALENE IN TISSUES FOLLOWING SUBCUTANEOUS INJECTION OF FENPROSTALENE IN PEG-400 (TRIAL 2)

<table>
<thead>
<tr>
<th>Time, d</th>
<th>Injection site</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.00</td>
<td>.74</td>
<td>2.25</td>
<td>.09</td>
<td>.15</td>
</tr>
<tr>
<td>3b</td>
<td>2.43</td>
<td>.18</td>
<td>.19</td>
<td>.12</td>
<td>.02</td>
</tr>
<tr>
<td>7</td>
<td>.94</td>
<td>.11</td>
<td>.10</td>
<td>.03</td>
<td>.01</td>
</tr>
<tr>
<td>14</td>
<td>.84</td>
<td>.05</td>
<td>.02</td>
<td>ND</td>
<td>.01</td>
</tr>
<tr>
<td>21b</td>
<td>.46</td>
<td>.04</td>
<td>.01</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>28</td>
<td>.31</td>
<td>.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

aExpressed as the radiochemical equivalent concentration of fenprostalene.
bValues are from one animal at 3 and 21 d. Duplicate animals were used at other time points.
cND indicates concentration was below practical detection limit of .01 ng/g.

Discussion

The process of absorption of an injected drug and the interaction of the drug with its vehicle as it affects absorption rate are complex phenomena, especially with respect to prostaglandins that are soluble in both aqueous and nonaqueous environments. In general, absorption of parenterally administered drugs can occur both by diffusion through pores in the capillary wall and by partitioning directly through the lipid bilayer of vascular endothelial cell membranes. The slower rate of absorption of fenprostalene from the PEG vehicle compared with the aqueous vehicle apparently is related to the increased viscosity of the PEG formulation. Experiments of Kakemi et al. (1972) indicated that when unionized drugs in water-soluble adjuvants, such as glycerin, propylene glycol or PEG-400, were injected, the drug and solvent were not separated but diffused together through pores in the capillary cell wall. Those investigators observed a close inverse relationship between absorption rate and viscosity of an injectable solution. Increased viscosity of PEG, however, does not provide a complete explanation for the observed decrease in absorption rate as discussed by Kent (1983). Decreased absorption rate of fenprostalene administered sc vs im would be expected due to decreased physical dispersion of the drug between muscle fibers and connective tissue and, consequently, decreased solubilization of the 2 ml injected dose. Likewise, a less abundant blood supply at the injection site also could be partially responsible for the decreased absorption rate of fenprostalene injected by the sc vs im route.

Regardless of its formulation and route of injection, the relatively long plasma t½ observed for fenprostalene compared with other prostaglandins is of particular interest. A t½ of 3 h after a maximum concentration at 15 min has been reported for im injection of 30 mg of PGF2α in an aqueous vehicle (Manns, 1975). The short t½ of the natural PGF2α in comparison with fenprostalene is due, at least in large part, to a rapid rate of metabolism. The first catabolic step, and most important with regard to biological inactivation of PGF2α, is oxidation of the 15-hydroxyl group to a 15-keto moiety (Hansen, 1976). This is followed by reduction of the Δ12-double bond yielding 15-keto-13,14 dihydro-PGF2α which is the main metabolite found in the plasma of calves (Kindahl, 1980). The carboxyl side chain also undergoes β-oxidation giving dinor and tetranor metabolites, the latter being major metabolites excreted in urine.

The chemical structure of fenprostalene differs from PGF2α in that both side chains were modified to reduce the rate of their catabolism. Elimination of carbon atoms 17 through 20 with the addition of a 16-phenoxy group is a substitution that inhibits oxidation of the 15-hydroxyl (Granström and Hansson, 1976) while greatly improving luteolytic potency compared with PGF2α (Binder et al., 1974; Crossley, 1975). Reeves (1978) reported that following im injection of only .5 mg of the 16-m-chlorophenoxy derivative (cloprostenol)
in citrate buffer, a peak plasma concentration averaging .45 ng eq/ml was observed at 15 to 30 min and then declined with a \( t_{1/2} \) of approximately 3 h. Excretion in urine was partly unchanged cloprostenol and partly the tetranor metabolite formed by \( \beta \)-oxidation of the unprotected carboxyl side chain (Bourne, as cited by Reeves, 1978).

Fenprostalene, with the \( \Delta^{4,5} \) allene group as well as the 16-phenoxy substitution, appears more resistant to metabolism than either PGF\(_2\alpha\) or cloprostenol. When solubilized in aqueous buffer and injected im, fenprostalene exhibited a \( t_{1/2} \) of 6.1 h, approximately double that reported for either PGF\(_2\alpha\) or cloprostenol after injection of the doses of those compounds recommended to induce luteolysis. Previously, Green et al. (1976) have reported that introduction of a double bond at the \( \Delta^5 \) position substantially increased resistance of the carboxyl side chain to \( \beta \)-oxidation and, consequently, increased the \( t_{1/2} \) of their PGF\(_2\alpha\) analog in the rat. Consequently, the chemical structure of fenprostalene coupled with its formulation in PEG provides a sustained-release prostaglandin analog displaying an extended \( t_{1/2} \), but no persistent tissue residue.

**Literature Cited**


