Metabolism of [^{14}C]Ceftiofur Hydrochloride in Swine after Intramuscular Injections

Maria G. Beconi-Barker,* Evan B. Smith, Thomas S. Arnold, Rex E. Hornish, Tom J. Vidmar, and Cathy L. Gatchell

Pharmacia & Upjohn Inc., Kalamazoo, Michigan 49007

Twelve mix-breed swine (26.5−42.5 kg) received three intramuscular doses of [^{14}C]ceftiofur hydrochloride at 24 h intervals. Three males and three females received 6.76 ± 0.83 mg of [^{14}C]ceftiofur free acid equivalents (CE)/kg body weight (bw)/day, while three males and three females received 4.41 ± 0.97 mg of CE/kg bw/day. All swine were euthanatized 12 h after the last dose. Most of the ^{14}C-residues (>80%) in tissues were associated with macromolecules. The rest of the ^{14}C-activity corresponded to desfuroylceftiofur (DFC)−cysteine. The type and percentages of metabolites present in the urine of swine were similar to those previously observed for ceftiofur sodium (Gilbertson et al., *J. Agric. Food Chem.* **1995**, *43*, 229−234), with DFC−cysteine and DFC-dimer being the major ones. In plasma, the major portion of the ^{14}C-activity (>85%) was associated with macromolecules. DFC−cysteine was the only detectable ^{14}C-component of the macromolecule-free plasma fraction. This study indicated that ceftiofur hydrochloride was absorbed in the same ionic form as ceftiofur sodium, acceding to the same primary metabolite DFC. The type of metabolites present in swine after intramuscular injection were the same, regardless of the salt administered.

Keywords: *Metabolism; ceftiofur hydrochloride; swine; EXCENEL sterile suspension*

INTRODUCTION

The metabolism of ceftiofur (NAXCEL/EXCENEL sterile powder), a cephalosporin antibiotic used solely in veterinary medicine, has been extensively studied (Jaglan et al., 1989, 1992; Beconi-Barker et al., 1995; Gilbertson et al., 1995). After intramuscular administration its metabolism is similar in all species studied. The metabolism of ceftiofur in animals differs from that of other cephalosporin antibiotics described in humans (Machinist et al., 1984; Nakayama et al., 1984; Neu and Shrinivasan, 1981; Patel and Kaplan, 1984), in that ceftiofur is metabolized before being excreted in urine. The half-life of ceftiofur in plasma is short, less than 10 min. Its primary metabolite is desfuroylceftiofur (DFC). During the cleavage of ceftiofur to DFC, furoic acid is liberated and generates metabolites found in the normal urine of humans eating cooked vegetables (Mrocheck and Rainey, 1972). Desfuroylceftiofur is further metabolized to the disulfides desfuroylceftiofur cysteine disulfide (DFC−cysteine) and desfuroylceftiofur glutathione and conjugates to macromolecules in both plasma and tissues.

Ceftiofur hydrochloride (EXCENEL sterile suspension) was recently approved by the Food and Drug Administration for the treatment of swine respiratory diseases. It is a ready-to-use product, as opposed to ceftiofur sodium (NAXCEL/EXCENEL sterile powder) which must be mixed with water prior to use. After several intramuscular administrations of ceftiofur hydrochloride to swine, the recovery of drug in excreta, drug distribution in tissues, and residue concentrations (Beconi-Barker et al., 1996) are similar to those observed after administration of ceftiofur sodium (Gilbertson et al, 1995). Over 70% of the ^{14}C-activity was excreted in the urine within a 12 h period after the last dose, while only 13% of the ^{14}C-activity was recovered in the feces (Beconi-Barker et al., 1996). The metabolism of ceftiofur hydrochloride in swine is described in this report.

EXPERIMENTAL PROCEDURES

Test Materials. Ceftiofur hydrochloride, radiolabeled with ^{14}C in the 2 position of the thiazole ring, was synthesized by R. E. Hornish of Pharmacia & Upjohn Inc. Within 1 week of dosing it was homogenized with micronized unlabeled ceftiofur HCl and vehicle (cottonseed oil base with 0.05% (w/v) phospholipon and 0.15% (w/v) sorbitan monooleate) by shaking in a Brinkman mixer mill. The resulting suspension contained 50 mg of [^{14}C]ceftiofur free acid equivalents (CE)/mL with an approximate specific activity of 1.5 μCi/mg. This suspension was dispensed into syringes, each containing the dose for one animal/day and frozen until dosing, since the stability of the radiolabeled drug at room temperature was unknown. Radiochemical purity was determined by high performance liquid chromatography (HPLC) to be >98%, using the HPLC conditions described in the dose preparation section.

Other Compounds. Ceftiofur-related metabolites used as standards for HPLC and MS were synthesized by C. L. Gatchell of Pharmacia & Upjohn Inc.

Animal Phase. The animal phase was previously described (Beconi-Barker et al., 1996). Briefly, twelve mixed-breed swine (Hampshire−Yorkshire−Landrace cross, 26.5−42.5 kg) were administered [^{14}C]ceftiofur HCl as intramuscular injections in the neck at 24 h intervals for three consecutive days. Three males and three females received 6.76 ± 0.83 mg of CE/kg body weight (bw)/day, while three males and three females received 4.41 ± 0.97 mg of CE/kg body weight. All swine were fed a feed concentrate and water *ad libitum*. Three days before treatment, the swine were placed in metabolism cages in a room artificially lighted (14 h of light/day), of ambient humidity and temperature (about 22 °C).

Dose Preparation. On the dosing day, the frozen doses were thawed and administered. The dose suspensions were assayed for total ^{14}C, in triplicate, by liquid scintillation counting (LSC). The potency and radiochemical purity of the dose suspensions were determined by HPLC with a radioactive monitor (RAM) as previously described (Beconi-Barker et al., 1995). Results of the radioactive assays demonstrated that the daily doses contained the intended amount of ^{14}C and were homogeneous.

Sample Collection. Urine and feces were collected separately and weighed at 24 h intervals, except for the third day, on which the time period varied from greater than 10 h to less

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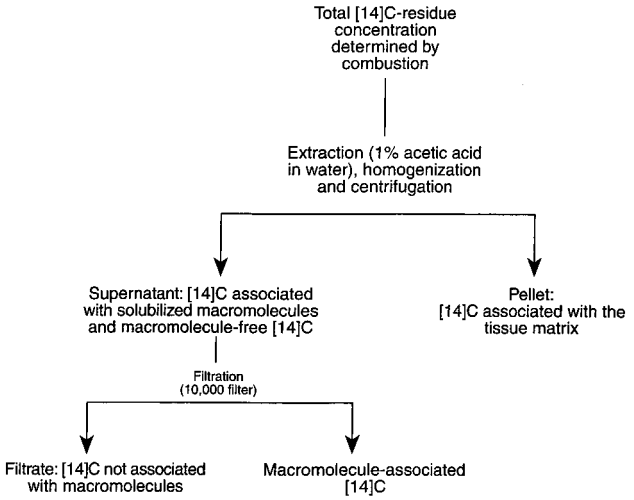

Figure 1. Scheme of drug extraction from swine tissues.

than 12 h. Blood was collected from the jugular vein at 0, 0.5, 1, 2, 3, 6, 12, and 24 h after each dose, except after the last dose (no 24 h collection). Plasma was harvested from an aliquot of blood and frozen until assayed. Approximately 500 g of the injection site, longissimus muscle and fat, and the whole kidney, liver, and lung were removed for analysis, ground, and divided into several 10 g aliquots after euthanasia. All aliquots were frozen at −20 °C until assayed.

Total Radioactivity Determination. Total radioactivity in tissues was determined by combustion analysis (Packard Tri Carb sample oxidizer) of triplicate weighed aliquots (∼0.5 g) followed by counting of ^{14}C as trapped CO_2 in a Packard Tri Carb Model 2000 LSC. Total radioactivity in feces was determined in the same manner after homogenizing with water (2 parts feces and 1 part water, about 0.5 g was combusted). Plasma (0.2 mL) and urine (0.2 mL) were counted directly by LSC after adding 15 mL of Instagel XF (Packard).

Preparation of Samples for Metabolite Profile. *Tissues.* Kidney (10 g), liver (20 g), and fat (10 g) from each swine were homogenized with 20 mL of 1% acetic acid and centrifuged at 48 000 RCF for 40 min. The volume of each supernatant was brought to 22 mL and the ^{14}C-activity recovered in each supernatant was determined from a 250 μL aliquot (in duplicate). The ^{14}C-activity not extracted in the supernatant was determined by difference between the ^{14}C-activity extracted in the supernatant and the total ^{14}C-activity measured after tissue combution (Figure 1). The remaining supernatant was transferred to individual Amicon Centriprep 10 units (Model 4304) and centrifuged at 3000 RCF for 2 h at 4 °C to separate the macromolecule-associated residues extracted from the matrix from the macromolecule-free residues. Each filtrate, which contained ^{14}C-activity not associated with macromolecules, was lyophilized. The dry residue was resuspended in 2 mL of 0.01M ammonium acetate (pH 5), for kidney and liver, and in a minimal volume of the same buffer for fat. Duplicate 50 μL aliquots of the reconstituted filtrate were analyzed for ^{14}C-activity. The remaining solution was analyzed by HPLC (system 1). The ^{14}C-activity in the macromolecule-associated fraction extracted from the matrix was determined by difference between the ^{14}C-activity in the supernatant after homogenization and the ^{14}C-activity in the macromolecule-free fraction (Figure 1). Insufficient activity was found in the muscle to allow radioactive determination following HPLC with the sensitivity of the instruments available.

Urine. Individual urine samples were filtered (Acrodisc filter, 0.45 μm). Duplicate 0.25 mL urine aliquots were analyzed for total ^{14}C-activity before and after filtering, to determine if there was any ^{14}C-activity loss due to filtration. After filtration, samples were analyzed by HPLC (system 2). Desfuroylceftiofur (DFC)-containing metabolites were identified by monitoring the disappearance of the radiolabeled peaks associated with these metabolites and the corresponding appearance of a radiolabeled peak corresponding to free DFC, which resulted from the cleaving action of excess dithioerythritol (DTE) in 0.1 M ammonium acetate (pH 8.7) at 50 °C for 2 h. The identities of radiolabeled peaks corresponding to DFC-containing metabolites were further confirmed by thermospray-mass spectrometry (MS) as follows: Radiolabeled peaks were collected from fractions obtained from repeated HPLC injections. Radioactive fractions with similar retention times were pooled and lyophilized. Each fraction was analyzed by thermospray-MS.

Plasma. Plasma samples (∼1.5 mL) obtained at 0, 0.5, 1, 2, 3, 6, 12, and 24 h after first dose and at 12 h after last dose (slaughter time) were placed in individual Amicon Centricon 10 units (Model 4206) and centrifuged at ∼5 °C overnight at 5000 RCF. The volume of each filtrate, which contained macromolecule-free residues, was accurately measured, and duplicate 100 μL aliquots were removed for total radioactivity determination. The remaining filtrate was transferred to an autosampler vial for HPLC analysis (system 2). The ^{14}C activity of the macromolecule-associated residues was estimated as the difference between the total activity found in plasma by combustion and the total activity found in filtrate.

Nature of the ^{14}C-Residues Associated with Macromolecules in Swine Plasma. The macromolecule-bound fractions obtained from plasma samples were incubated with excess DTE to liberate the ^{14}C-residues as follows. Pooled macromolecule fractions were generated from plasma obtained 1 h after the first dose (observed maximum ^{14}C-concentration) and 12 h after last dose (slaughter time). Each pooled macromolecule fraction was divided into four aliquots (∼500 mg each). Three milliliters of 4% dithioerythritol (DTE) in borate buffer (w/v) was added to each aliquot. Two of the four aliquots per sampling time were incubated at 50 °C for 15 min, and the remaining two were incubated at room temperature for 2 h. After incubation, 3 mL of acetonitrile was added to precipitate the macromolecules, and vials were vortexed and centrifuged at 48 000 RCF for 30 min. The supernatant, containing ^{14}C-residues liberated from the macromolecules, was transferred to a clean vial and the solvent was evaporated at 35 °C under a stream of nitrogen until ∼0.5 mL remained. The remaining aliquot was acidified with acetic acid (3 drops), vortexed, transferred to a 1.5 mL centrifuge tube, and centrifuged for 10 min at maximum speed in a benchtop Sorvall MC 12C centrifuge. The supernatant was transferred to an autosampler vial for HPLC analysis (system 2).

Nature of the Macromolecule-Free ^{14}C-Residues in Swine Plasma. Three pooled macromolecule-free fractions were generated for the sampling times for which maximum macromolecule-free residues were observed. These corresponded to samples between 1 and 3 h after the first dose. Samples were lyophilized, resuspended in 1 mL of 8% DTE in borate buffer (w/v), and incubated for 2 h at 50 °C. A 500 μL aliquot of each solution was analyzed by HPLC (system 2).

Instrumental Analysis. *Thermospray Mass Spectrum.* The thermospray interface was a Nermag/Vestec system/controller (Vestec Corp., Houston, TX), operated at various source and vaporizer temperature settings and repeller voltages. The mass spectrometer was a Nermag resolver R 10-10 L quadrupole instrument, 2000 amu, fitted with the Nermag/Vestec thermospray source and probe, set to operate in the $Ions^+$ mode. The MS controlling software/hardware was the PC (OS/2) based Teknivent Vector/Two GC/LC/MS workstation, V1.4, Teknivent, 149 Weldon Parkway, Maryland Heights, MO.

During the analysis of samples, the acquisition was set in the scan mode from m/z 150 to m/z 900 with a scan rate of 0.5 scans/s.

HPLC Systems. The HPLC system consisted of a Waters 600 E powerline multisolvent delivery system, controller and pump equipped with a 717 Plus autosampler and a 996 photo diode array detector monitored at 254 nm, and a Radiomatic FLO-ONE/Beta Model A-500 radioactive flow detector and processing software.

HPLC Conditions. System 1. A Burdick and Jackson (250 × 4.6 mm), HLD OC5 octyl, 5 μm column was used. The mobile phases were the following: A consisted of 0.01 M ammonium acetate (pH 5.0) and B of 6:4 (v/v) methanol:water.

Table 1. Distribution of [^{14}C]Ceftiofur Related Residues in Swine Tissue Fractions

tissue	dose level (mg/kg)	^{14}C-residue concentration[a] (μg/g tissue)	^{14}C-activity extracted from matrix[b] (%)	^{14}C in soluble macromolecules[b,c] (%)	macromolecule-free ^{14}C[b] (%)
kidney	6.76	10.68 ± 2.68	44.2	31.3	7.4
	4.41	6.33 ± 1.75	59.9	38.9	11.2
liver	6.76	2.64 ± 0.46	49.6	39.0	7.2
	4.41	1.79 ± 0.35	52.8	38.6	7.3
fat	6.76	2.45 ± 0.63	25.4	19.6	5.3
	4.41	1.34 ± 0.40	34.0	28.4	6.0

[a] Determined by combustion of ^{14}C. [b] Percentage of total. [c] The determination of ^{14}C in soluble macromolecules carries a degree of uncertainty due to the high viscosity of the samples.

The gradient was linear from 0 to 20% B from 0−10 min, remained isocratic (20% B) from 10 to 35 min, increased linearly to 100% B from 35 to 45 min, and remained isocratic (100% B) from 45 to 55 min. The flow was 1 mL/min. The column was equilibrated with the starting conditions with a 1.5 mL/min flow for 20 min before the next injection.

System 2. A Keystone Scientific (250 × 4.6 mm), BDS Hypersil C18, 5 μm column was used. The mobile phases were the following: A consisted of 0.1% (v/v) TFA in water and B of 0.1% (v/v) TFA in acetonitrile. The gradient was isocratic for 5 min (0% B), increased linearly from 0 to 35% B from 5 to 40 min and from 35 to 50% B from 40 to 50 min. The flow was 1 mL/min. The column was washed for 10 min (80% B, flow 1.5 mL/min) and equilibrated with the starting conditions with a 1.5 min/mL flow for 25 min before the next injection.

In this paper, all data are reported as mean and standard deviation.

RESULTS AND DISCUSSION

Tissue Metabolite Profile. *Nature of Kidney and Liver Metabolites.* The ^{14}C-activity recovered in the kidney and liver extracts represented between 40 and 60% of the total ^{14}C-residue concentration obtained by combustion (Beconi-Barker et al., 1996), for both dose levels (Figure 1, Table 1). The remaining ^{14}C-activity was associated with nonsolubilized macromolecules and was not extracted from the kidney matrix under the extracting conditions used. The extracted fraction consisted primarily of ^{14}C-residues associated with solubilized macromolecules (between 30 and 40% of the total radioactivity for both tissues and dose levels) with a small fraction of macromolecule-free residues (less than 12% of the total radioactivity for both tissues and dose levels).

HPLC-Radioactive monitoring (HLC-RAM) analysis (system 1) of the kidney and liver macromolecule-free fractions of all animals indicated the presence of one major radioactive peak (Figure 2A). This peak corresponded by retention time to DFC−cysteine. Incubation of a pooled sources of macromolecule-free kidney and liver extracts with the reducing agent DTE yielded only one radiolabeled peak, which eluted at the same retention time as DFC. This indicated that all ceftiofur-related metabolites found in the macromolecule-free swine kidney extract were conjugates of DFC (Figure 2B).

Nature of Fat Metabolites. The recovery of ^{14}C-activity and its distribution between the macromolecule fraction and the macromolecule-free fraction in swine fat followed a similar pattern to the one observed for kidney and liver, but were numerically lower. The ^{14}C-activity recovered in the fat extracts represented, on the average, between 25 and 35% of the ^{14}C-residue concentration determined by combustion (Beconi-Barker et al., 1996) for both doses. More than 65% of the ^{14}C-activity was associated with nonsolubilized macromolecules and was not extracted from the fat matrix under the extracting conditions used. The extracted fraction

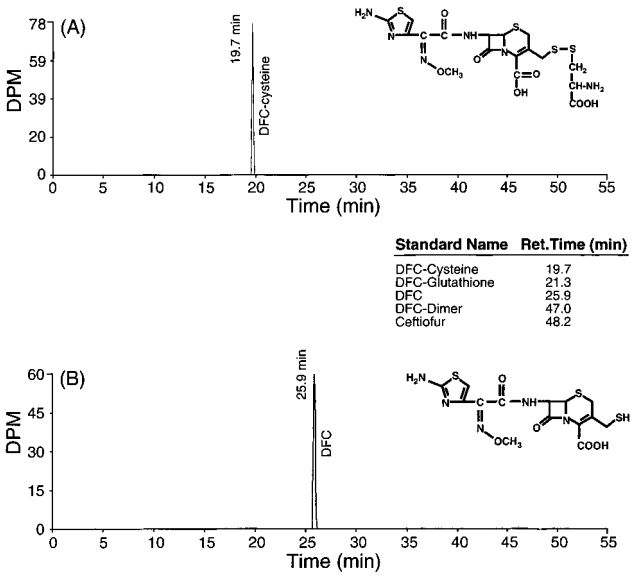

Figure 2. (A) Representative HPLC ^{14}C-chromatogram of macromolecule-free swine kidney extract. (B) Representative HPLC ^{14}C-chromatogram of macromolecule-free swine kidney extract after treatment with the reducing agent dithioerythritol. Chromatograms were obtained using HPLC system 1.

consisted primarily of ^{14}C-residues associated with solubilized macromolecules with a small fraction of macromolecule-free residues (Figure 1, Table 1).

Only one major radioactive peak was detected upon HPLC analysis (system 1) of the pooled fat macromolecule-free fraction, which eluted at the retention time of DFC−cysteine. Incubation of the pooled macromolecule-free fat extract with DTE yielded DFC as the only radiolabeled metabolite, indicating that in this fraction all ceftiofur-related metabolites found were conjugates of DFC.

Urine Metabolite Profile. Total radioactive analysis of urine samples before and after passing through the Acrodisc filters indicated there was no loss of radioactivity due to filtering. To check that there was no radioactivity loss due to the filtering process, the difference (Δ) between the DPM obtained before and after filtering was calculated for each sample wihtin each dose and collection time. The resulting Δ values were used to statistically test the hypothesis that the average Δ is equal to zero using a paired *t*-test.

Upon HPLC analysis (system 2) of the urine samples, five radioactive peaks were detected (Figure 3A). The radioactive peak eluting before 10 min was attributed to a mixture of polar metabolites of ceftiofur due to the early elution time from the C-18 HPLC column. The retention time of peak 2 (19.52 ± 0.33 min) did not correspond to the retention time of any of the known ceftiofur metabolites. The fragmentation pattern resulting from thermospray-MS analysis of this peak did not have the characteristic ions of the intact DFC

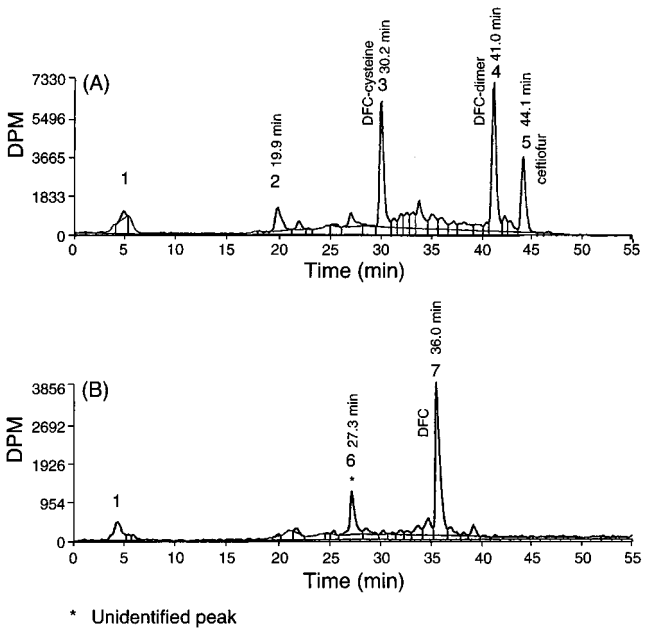

Figure 3. (A) Representative HPLC 14C-chromato gram of swine urine. (B) Representative HPLC 14C-chromatogram of swine urine after treatment with the reducing agent dithioerythritol. Chromatograms were obtained using HPLC system 2.

moiety. The characteristic $[M + H]^+$ ions of the intact DFC moiety after thermospray-MS analysis are 326, 352, 386, and 431. Preliminary characterization indicated that it probably corresponded to a polar metabolite of ceftiofur devoid of the intact β-lactam ring. Note that with the thermospray technique, the molecular ions of ceftiofur and related metabolites are very weak. In addition, the concentration of this analyte was low. Complete characterization of this metabolite is in progress. The retention times of peaks 3 (30.2 ± 0.2 min), 4 (41.0 ± 0.2 min), and 5 (44.1 ± 0.2 min) corresponded to the retention times of DFC−cysteine, DFC-dimer, and ceftiofur, respectively. Their identity was confirmed by thermospray-MS. All other radioactive components present in the chromatograms represented, individually, on average less than 5% of the total radioactivity in urine and were attributed to a mixture of ceftiofur-derived isomers and artifacts of the HPLC solvent system.

The radioactive signals due to peaks 2−5 disappeared upon treatment of the samples with the reducing agent DTE, while DFC (peak 6) was the major radiolabeled metabolite (Figure 3B). This indicated cleavage of the disulfide bond of the conjugates to DFC, the biologically active metabolite of ceftiofur which possesses an intact β-lactam ring. A second peak of minor intensity (peak 7, 13.2% of the total radioactivity in the incubated extract) with a retention time of 27.3 min was also observed. This peak eluted early, and its retention did not correspond to that of any of the known ceftiofur-related metabolites. It is believed that peak 7 was a product of the unidentified peak 2 due to DTE treatment.

The percentages of 14C-metabolites found in the urine of swine dosed at 4.41 or 6.76 mg of CE/kg bw at 24, 48, and 60 h after the first dose are listed in Table 2. There was large variability among animals. The average percentage of polar metabolites ranged between 6.9 and 17.2% and between 3.5 and 10.4% for animals dosed with 4.41 and 6.76 mg of CE/kg bw, respectively. The percentage of the unknown metabolite ranged between

Table 2. Percent of Various Metabolites Found in the Urine of Swine following Multiple Intramuscular Injections of Ceftiofur HCl

	average (SD)				
time (h)[a]	% polar	% unk1	% DFC−cyst	% DFC-dimer	% parent
	Dose Level = 4.41 mg of CE/kg bw				
24	17.2 (2.5)	10.8 (5.7)	33.6 (6.2)	24.3 (12.3)	14.1 (4.2)
48	14.1 (9.9)	11.5 (6.4)	38.3 (12.2)	19.6 (5.6)	16.5 (5.7)
60[b]	6.9 (2.4)	5.4 (1.6)	32.3 (6.9)	27.1 (7.8)	28.34 (4.5)
	Dose Level = 6.76 mg of CE/kg bw				
24	7.7 (4.1)	5.3 (2.2)	31.0 (7.2)	37.7 (10.3)	18.2 (6.3)
48	10.4 (3.2)	8.9 (3.2)	29.9 (3.1)	32.7 (8.6)	18.1 (4.2)
60[b]	3.5 (2.6)	5.7 (2.8)	27.0 (3.7)	40.9 (8.3)	22.9 (5.7)

[a] Hours after first treatment (doses occurred at 0, 24, and 48 h).[b] Collections 12 h after last treatment.

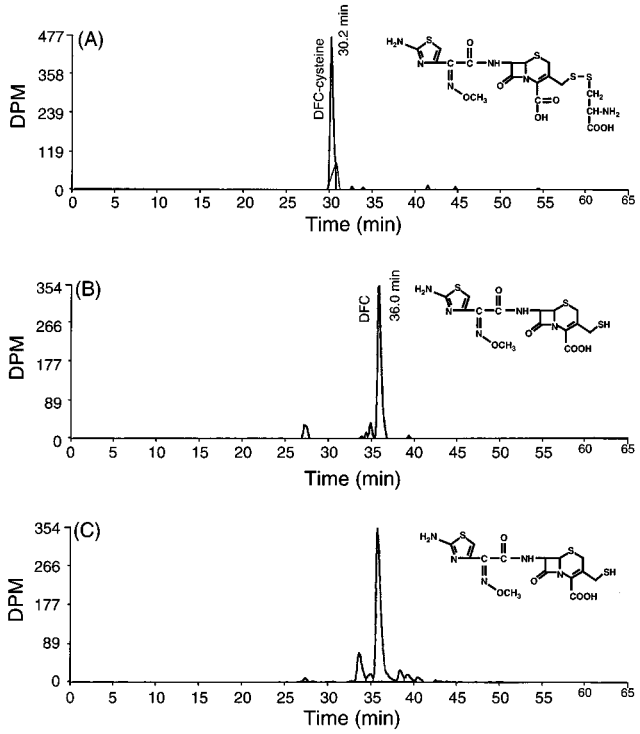

Figure 4. (A) Representative HPLC 14C-chromatogram of the macromolecule-free fraction of swine plasma. (B) Representative HPLC 14C-chromatogram of the macromolecule-free fraction of swine plasma after treatment with the reducing agent dithioerythritol. (C) Representative HPLC 14C-chromatogram of the macromolecule-associated fraction of swine plasma after treatment with the reducing agent dithioerythritol. Chromatograms were obtained using HPLC system 2.

5.4 and 11.5% and between 5.3 and 8.9% for the 4.41 and 6.76 mg of CE/kg bw dosing groups, respectively. For swine dosed at 4.41 mg of CE/kg bw, DFC−cysteine was the most abundant urinary metabolite, representing 33.6−38.3% of the total radioactivity in urine for the 24 and 48 h urine collections. The second most abundant urinary metabolite for those collection times was DFC-dimer (19.6−24.3%), followed by unmetabolized ceftiofur (14.1−16.5%). At 12 h after the last dose, DFC−cysteine was still, on the average, the major urinary metabolite (32.3 ± 6.9%), while unmetabolized ceftiofur (28.3 ± 4.5%) and DFC-dimer (27.1 ± 7.8%) were excreted in similar amounts. For swine dosed at 6.76 mg of CE/kg bw, DFC-dimer was the most abundant urinary metabolite, representing 32.7−37.7% of the total radioactivity in urine for the 24 and 48 h urine collections, respectively. The second most abundant urinary metabolite for those collection times was DFC−

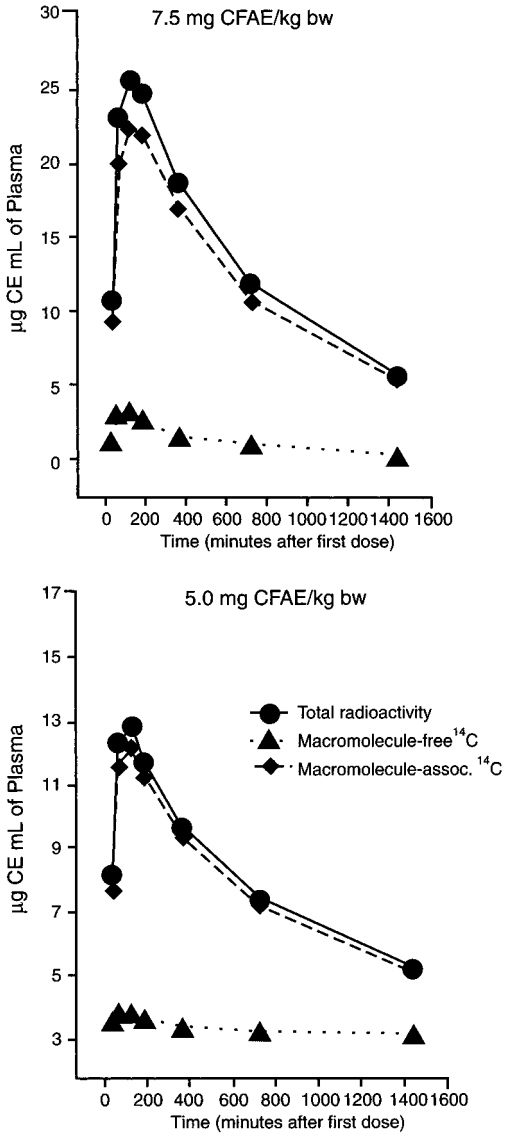

Figure 5. ^{14}C-residue concentrations in plasma.

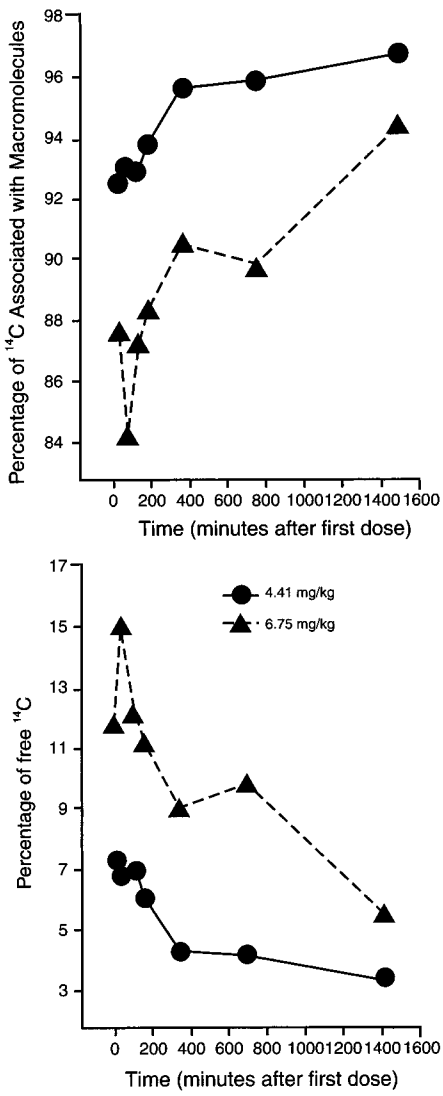

Figure 6. Percentage (percentage of total ^{14}C-residues in plasma) of macromolecule-free (A) and macromolecule-associated (B) ^{14}C-residues in plasma vs time.

cysteine (29.9−31.0%), followed by unmetabolized ceftiofur (18.1−18.2%). At 12 h after the last dose, DFC-dimer was on the average the most abundant metabolite (40.9 ± 8.3%), followed by DFC−cysteine (27.0 ± 3.7%) and unmetabolized ceftiofur (22.9 ± 5.7). There was a trend for the percentage of DFC-dimer to be higher for the 6.71 mg of CE/kg bw dosing group; this is probably of no biological significance, since the dimer forms after the DFC has been released from the protein to clear in the urine. The percentages of the rest of the radiolabel metabolites found in urine were similar between both doses, and did not appear to be dose dependent. A metabolite profile of feces was not obtained since it was previously shown (Gilbertson et al. 1990) that ceftiofur and related metabolites are readily degraded to microbiologically inactive products by substances or organisms present in feces.

Plasma Metabolite Profile. Only one radioactive peak was observed in all macromolecule-free plasma fractions (Figure 4A). This peak corresponded by retention time to DFC−cysteine (Table 1) and disappeared upon treatment with the reducing agent DTE, yielding DFC (Figure 4B). Incubation of the macromolecule plasma fraction with DTE also yielded DFC as the major ^{14}C-residue.

Within the first dosing period, plasma samples obtained at 2 h contained the maximum concentration of ^{14}C-residues. At this time, maximum concentrations of ^{14}C-residues were also observed in the macromolecule-free plasma fraction and in the fraction containing residues associated with macromolecules (Figure 5). After the ^{14}C residues reached a maximum concentration in plasma, the percentage of ^{14}C-residues associated with macromolecules increased, on the average, over time from 92.63 to 96.63% and from 88.11 to 94.48% for swine dosed at 4.41 and 6.76 mg of CE/kg bw, respectively (Figure 6). Concurrently, the percentage of ^{14}C-residues not associated with macromolecules decreased from 7.37 to 3.37% and from 11.89 to 5.51% for swine dosed at 4.41 and 6.76 mg of CE/kg bw, respectively.

CONCLUSIONS

When swine were administered either ceftiofur sodium or ceftiofur hydrochloride intramuscularly, most of the dose was excreted via the urine (>60%), followed by feces (~10−13%) (Gilbertson, el al., 1995; Beconi-Barker et al., 1996). Dose recoveries from urine and feces were similar among doses and between salts. In addition, the distribution of the drug in tissues followed

the same pattern regardless of the ceftiofur salt or dose level administered. Excluding the injection sites, the highest residue concentrations within each animal were always found in the kidneys, followed by the liver, fat, and muscle. For similar doses, from different salts, tissue residue concentrations were similar, suggesting similar availability of the drug.

In this study, most of the ^{14}C-residues found in swine kidneys, liver, and fat remained with the tissue following extraction or were retained with the macromolecule-associated fraction after filtration. Thus, most of the ^{14}C-activity (>80%) was bound to macromolecules. Most of the ^{14}C-activity detected in the macromolecule-free fraction corresponded to DFC−cysteine. In plasma, most of the ^{14}C-activity (>85%) was associated with macromolecules. This was true even at 20 min after dose administration. The ^{14}C-residue associated with the macromolecule fraction was DFC. In the macromolecule-free plasma fraction, DFC−cysteine was the only detectable ^{14}C-component. It was previously shown (Beconi-Barker et al., 1996) that over 70% of the dose is released in urine at 12 h after dosing, with a total accountability of over 90% of drug in excreta. No macromolecule-associated residues are found in the urine. For this to occur, DFC has to be liberated from the macromolecules. Probably in the bladder, DFC will find other DFC molecules to form the DFC-dimer.

The type and percentages of metabolites present in the urine of swine were similar to those previously observed for ceftiofur sodium (Gilbertson et al., 1995). DFC−cysteine and DFC-dimer were the major metabolites found in the urine of swine administered either ceftiofur hydrochloride or ceftiofur sodium (∼27−41% of the total ^{14}C-residues eluting from the HPLC), followed by unmetabolized ceftiofur (∼18−30%). Polar metabolites constituted individually <10% of the total ^{14}C-residues at 12 h after the last administration.

After intramuscular injection of ceftiofur to swine, the dose recovered in excreta, the drug distribution in tissues, and residue concentrations were similar for a given dose level, regardless of the ceftiofur salt administered (Gilbertson et al., 1995; Beconi-Barker et al., 1996). This study indicated that ceftiofur hydrochloride is absorbed in the same ionic form as ceftiofur sodium (the extent of ionization is unknown), acceding to the same primary metabolite DFC. The type of metabolites present in swine after intramuscular injection are the same, regardless of the salt or dose level administered.

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