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Comparison of Concentrations of Rifampin and a New Rifamycin Derivative, DL 473, in Canine Bone

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Constant-infusion experiments were performed in 14 dogs to determine the penetration into bone of rifampin and a new C-3 substituted rifamycin, DL 473. The drugs were assayed in cortical bone and medulla from tibia-femur and cortical and cancellous bone from rib. After identical dosage, the concentrations of DL 473 appeared to be higher, except in the medulla, although the serum concentrations of rifampin were almost twice as high as those for DL 473. The concentrations of both drugs in all bone areas were several times higher than their minimum inhibitory concentrations against pathogenic *Staphylococcus aureus*.

DL 473 [3-(4-cyclopentyl-1-piperazinyl)-iminomethyl-rifamycin] is a new highly lipid-soluble analog of rifampin with an antibacterial spectrum similar to that of rifampin. The in vitro activity of DL 473 against *Mycobacterium tuberculosis* has been found to be superior to that of rifampin, whereas rifampin appears to be two to four times stronger against almost all other susceptible microorganisms, including *Staphylococcus aureus*. The minimum inhibitory concentration of rifampin against *S. aureus* is 0.006 to 0.012 μg/ml, and that of DL 473 is 0.025 μg/ml (personal communication, DOW-LEPETIT, Milan, Italy). The purpose of this study was to investigate the ability of DL 473 and rifampin to concentrate in bone tissue of dogs.

Fourteen male mongrel dogs, weighing from 13.2 to 37.0 kg each, were intravenously anesthesized with sodium thiopenthal. Rifampin and DL 473 (supplied by DOW-LEPETIT Corp., Midland, Mich.) were each administered intravenously to seven dogs. Both antibiotics were given as bolus injections of 15 mg/kg of body weight, followed by a constant infusion of 1.88 mg/kg per h for 4 h. Arterial blood samples were drawn every 30 min throughout the experiment.

Bone samples were taken with a rongeur every hour from the tibia or the femur, starting 1 h after bolus injection. Sampling began distally and moved proximally throughout the experiment. Specimens of medulla were aspirated through holes in the cortex. Approximately 1 cm of rib was removed through a small incision in the chest wall, split, and divided into cancellous and cortical bone tissue. The cortical bone samples were scraped clean of periosteum and cancellous bone tissue and washed in 1 ml of saline for 5 s. Bone sampling was completed approximately 15 min after the corresponding serum sample was taken. Samples were frozen 1 to 2 weeks at −20°C before final analysis, so that only minor drug degradation occurred (4).

Cancellous and cortical samples were immersed in liquid nitrogen, and the resulting brittle tissue was hammered to a fine powder between two steel plates precooled in dry ice. Phosphate-buffered saline (0.1 M; pH 4.5) was added to the powder in an amount equal to twice the weight of the powder. These samples were agitated and frozen. Twenty-four hours later, the samples were thawed, agitated, and left overnight at 4°C before bioassay. Medulla specimens were homogenized in 0.1 M phosphate buffer at pH 4.5 and left overnight at 4°C. Concentrations of rifampin and DL 473 in the supernatants were assayed microbiologically by a disk diffusion method, employing *Sarcina lutea* ATCC 9341 on Seed agar containing 3% 0.1 M phosphate buffer, pH 4.5. All bone tissue samples were assayed against a buffer standard, since a series of recovery experiments revealed recoveries of 86 to 103% and 82 to 114% for DL 473 and rifampin, respectively, after bone powder was added to the standards. Serum samples were assayed by the same bioassay method as described above, using pooled dog serum for standards.

As shown in Table 1, steady-state conditions were reached after 1 h of constant infusion. The data from all sampling times in the DL 473 and rifampin series were pooled and analyzed statistically, employing the Mann-Whitney rank sum test and considering P values of less than 0.05 significant. Rifampin (median, 25 μg/ml; range, 13 to 41) had a significantly higher ($P < 0.0001$) serum concentration than DL 473 (median, 15

TABLE 1. Concentrations (median and range) of rifampin and DL 473 in canine bone tissue and serum during constant infusion

Bone tissue	Concn (μg/g) at following time (h)[a]:							
	1		2		3		4	
	Rifampin	DL 473	Rifampin	DL 473	Rifampin	DL 473	Rifampin	DL 473
Medulla (tibia-femur)								
Median	2.2	4.5	6.4	3.0	6.8	4.6	6.2	3.5
Range	0.9–6.0	1.1–11.5	1.1–8.0	1.7–5.8	1.3–8.5	1.3–16.5	0.3–9.5	1.8–11.3
Cortical bone (tibia-femur)								
Median	0.3	0.8	0.5	0.9	0.4	0.9	0.4	1.6
Range	0.2–1.3	0.3–1.7	0.2–1.3	0.5–5.2	0.2–2.0	0.7–3.4	0.3–1.8	0.6–3.2
Cortical bone (rib)								
Median	1.7	2.4	1.5	2.7	1.4	2.6	1.4	2.1
Range	0.9–3.1	1.7–4.0	0.6–3.1	1.9–3.7	0.8–2.7	2.0–5.5	0.8–3.4	1.5–3.1
Cancellous bone (rib)								
Median	1.1	4.6	1.7	3.9	1.1	4.6	1.2	4.9
Range	0.5–7.3	2.6–6.7	1.0–8.2	1.9–6.0	0.4–7.5	3.5–9.2	0.5–6.8	3.1–7.9

[a] The number of dogs given the drugs was seven for both rifampin and DL 473. In serum, the concentrations (in micrograms per milliliter) for rifampin and DL 473, respectively, were as follows: 1 h, 24 (mean), 13 to 31 (range) and 19, 10 to 25; 2 h, 26, 18 to 38 and 17, 10 to 22; 3 h, 24, 20 to 41 and 15, 11 to 25; 4 h, 25, 20 to 40 and 14, 10 to 18.

μg/ml; range, 10 to 25), implying different pharmacokinetic properties. Nevertheless, DL 473 obtained significantly higher concentrations than rifampin in cortical bone (tibia-femur) (median, 1.0 μg/g and range, 0.3 to 5.2, versus median, 0.4 μg/g and range, 0.2 to 2.0; $P < 0.0001$), cortical bone (rib) (median, 2.6 μg/g and range, 1.5 to 5.5, versus median, 1.4 μg/g and range, 0.6 to 3.4; $P < 0.0001$), and cancellous bone (rib) (median, 4.6 μg/g and range, 1.9 to 9.2, versus median, 1.2 μg/g and range, 0.4 to 8.2; $P < 0.0003$). No difference was encountered when medulla (tibia-femur) was considered. However, when the ratio of medulla to serum concentration was analyzed, DL 473 (median, 0.24; range, 0.06 to 1.10) was found to be superior ($P < 0.03$) to rifampin (median, 0.18; range, 0.01 to 0.44). The blood contamination in the bone biopsies was estimated with Hemoccult slides (Smith Kline Diagnostics, Sunnyvale, Calif.). The blood concentration was <1% in both cortical and cancellous bone.

Assays of antibiotics in bone are being performed with increasing frequency. However, many methodological problems still persist, and bone tissue/serum ratios of many antibiotics vary over a 10-fold range (6). This may be explained by calculation of tissue/serum ratios after a single injection of an antibiotic without consideration of the kinetics between the compartments. Steady state, as attempted by constant infusion experiments, must therefore be preferred. Several investigators have pulverized bone specimens and extracted the antibiotic in sterile water, buffer, or serum (1–3, 5). One study suggests the importance of particle size in the bone powder, in contrast to another study (1) which finds good correlation between the concentrations of clindamycin in bone obtained by pulverization-extraction and that obtained by applying small pieces of bone (0.01 to 0.5 g) directly to the surface of agar plates.

Although the median serum concentration of rifampin was approximately two times the median serum concentration of DL 473, the median concentration of DL 473 found in cancellous bone was more than triple that of rifampin, supporting our findings of only minimal blood contamination of the bone biopsies.

DL 473 demonstrated a statistically significant higher tissue/serum ratio than rifampin. This may be explained by a better ability of DL 473 to penetrate into bone, possibly caused by its greater lipid solubility or by a saturation of bone tissues at lower serum levels than those achieved in this study. A dose-response curve would elucidate this.

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