Br. J. Pharmac. Chemother. (1967), **30**, 627–630.

LINCOMYCIN SERUM AND BONE LEVELS IN THE RAT

BY

H. J. KESSLER, P. NAUMANN AND J. KEMPF

From the Institute for Medical Microbiology and Serology, University of Hamburg, Germany

(*Received April* 18, 1967)

The successful treatment of acute and chronic osteomyelitis with the antibiotic lincomycin (Upjohn) has been the subject of several recent reports (Holloway, Kahlbaugh & Scott, 1963; Geddes, Sleet & McMurdoch, 1964; McLeod, Ross, Ozere, Digout & Van Rooyen, 1964; Grondin, St. Martin & Potvin, 1965; Holloway & Scott, 1965; Kaplan, Chew & Weinstein, 1965; McMillan, McRae & McDougall, 1967). While the investigations of Myer & Lewis (1963) and Ma, Lim & Nodine (1963) have elucidated the general pharmacokinetics of lincomycin, knowledge about the concentration in bone is incomplete and more information would be of interest.

In osteomyelitis patients treated with lincomycin, Holloway, Kahlbaugh & Scott (1963) found concentrations ranging from 1.1 to 6.6 μg/g of bone. Simultaneous determinations of serum concentrations showed these to be higher than those in bone. As calculated from the data of the authors, the amount found in bone was 13.3 to 18.3% of that in serum.

Conflicting results were published by Grady & Stern (1965). In their study in rats, the bone concentration regularly exceeded the serum concentration after a single oral administration of 100 mg/kg of an aqueous lincomycin solution. However, no appreciable binding of lincomycin to bone (for example by chelate formation as with tetracycline) was noted.

The experiments described below were carried out to investigate further the concentrations of lincomycin in serum and bone in rats.

METHODS

Normal albino rats with an average weight of 150 g were used. Each was given a single dose of 20 mg/kg lincomycin HCl. To avoid the wide variation of serum concentration commonly observed after oral administration because of variability in absorption, the dose was injected intramuscularly in aqueous solution. Animals were killed at intervals after the dose and the lincomycin content of serum and bone assayed. In the second part of the study 10 rats were treated with repeated injections of 20 mg/kg 12-hourly for 6 days and on the morning of the seventh day. Antibiotic concentrations in bone and serum of 2 animals were determined ½, 1, 2, 4 and 6 hr after the last injection. For all experiments lincomycin hydrochloride was used, provided in vials containing 20 mg. (Upjohn, Lot 14, 231–3).

The concentration of the antibiotic in serum and bone was determined by means of the agar diffusion technique (cup plate method). The agar was autoclaved and then adjusted to pH 7.8.

Sarcina lutea ATCC 9341 served as test organism, the inoculum containing about 10^5 organisms/ml. agar. After a pre-diffusion time of 4 hr at room temperature, the test trays were incubated for 20 hr at 37° C. The zones of inhibition were measured and the concentration of the antibiotic was calculated by comparison with a standard curve on semilogarithmic graph paper.

Determination of serum levels

The reference standard was diluted with pooled serum of untreated rats. The serum employed had no antibacterial activity against the test organism. The lower limit of sensitivity for the detection of lincomycin in serum was 0.2 μg/ml. For each assay the serum of two animals was pooled.

Determination of bone levels

Immediately following cardiac puncture to obtain serum, the hindlegs of 2 rats were severed and muscles and periosteum removed. The bones (diaphyses of tibia and femur) were then split lengthwise and the marrow was scraped out with a sharp spoon. Any remaining marrow was carefully wiped off with cellulose tissue. To avoid heat-inactivation of the antibiotic, the bones were carefully and slowly milled to a fine powder in an ordinary coffee mill manually and weighed. The powder was extracted for 20 hr at 4° C with phosphate buffer, pH 7.8. To each gram of powder 2 ml. of the buffer were added. After centrifugation (3,000 r.p.m. for 5 min), the supernatant was utilized for the microbiological assay. In all determinations the bones of two animals were pooled.

Bone powder was also suspended for 20 hr in lincomycin solution (10 μg/ml.). No differences were seen when the activity of this solution was compared with a newly prepared standard in phosphate buffer only. It was concluded that under the conditions of the extraction the antibiotic lost no activity and was not bound irreversibly to the bone. The lower limit of sensitivity in determining lincomycin was 0.3 μg/ml. bone extract, equivalent to 0.6 μg/g bone powder.

RESULTS

The serum and bone concentrations found are presented in Table 1.

Serum levels

Thirty minutes after the intramuscular injection of 20 mg/kg lincomycin, an average serum concentration of 9.72 μg/ml. was found. After that time, the level declined steadily and 6 hr after the injection lincomycin could no longer be detected in serum. At this time the concentration of lincomycin was less than 0.2 μg/ml. in all experimental animals. Based on these results the half-life of lincomycin in serum was calculated as 38.4 min.

Bone levels

In all determinations the bone levels of lincomycin, expressed in μg/g bone were lower than the corresponding serum levels (Table 1). Thirty minutes after the injection a concentration of 3.5 μg/g bone was found. Subsequent determinations showed that the half-life of lincomycin in bone was practically identical with that in serum. Two hours after the injection the bone level had decreased below 0.6 μg/g and could, therefore, no longer be determined. This finding explains the lack of accumulation of lincomycin despite continued administration of doses of 20 mg/kg at 12 hr intervals. Even on the 7th day of treatment the levels in serum and bone were about the same as those found after a single injection.

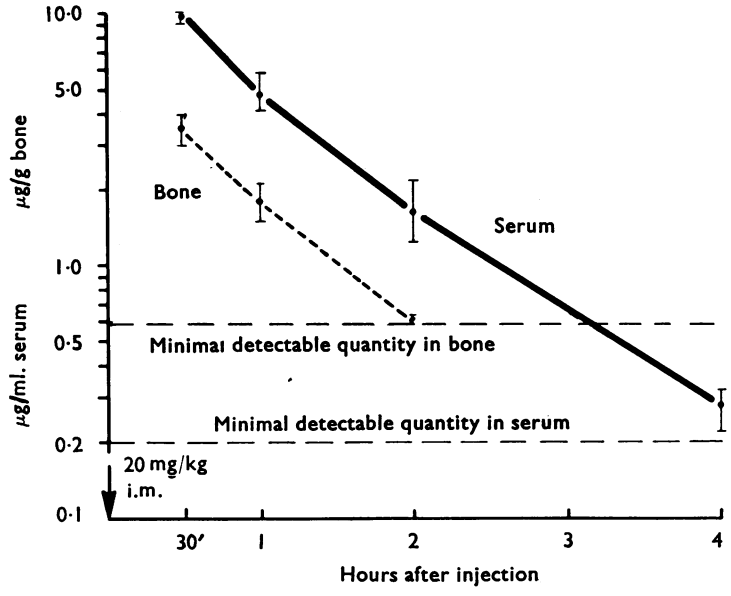

Fig. 1. Serum- and bone-levels of lincomycin in rats following single intramuscular injection of 20 mg/kg (average 32 animals). The vertical bars indicate arithmetic mean value and maximum and minimum measured antibiotic activity.

TABLE 1

SERUM AND BONE LEVELS AFTER SINGLE INTRAMUSCULAR INJECTION OF 20 MG/KG OF LINCOMYCIN

Arithmetic means with standard of errors of a total of 40 rats; for each point average data of 8 animals.

Time after injection (hr)	No. of animals	Serum level (μg/ml.)	Bone level (μg/g)	Bone level as % of serum level
0·5	8	9·72±0·23	3·50 ±0·50	36·0
1·0	8	4·84±0·29	1·80 ±0·30	37·2
2·0	8	1·65±0·17	0·603±0·023	36·5
4·0	8	0·28±0·02	<0·60	—
6·0	8	<0·20	<0·60	—

DISCUSSION

Grady and Stern (1965) reported that 15 min after a single oral dose of 100 mg/kg lincomycin to rats, the level in bone (14 μg/g) was 1550% of the level in serum 0.9 μg/ml.). After 4.5 hr, the bone levels were still 340 to 260% higher than the serum levels. The finding of such extraordinarily high bone levels of lincomycin as early as 15 min after oral administration is very surprising, considering the slow absorption (as demonstrated in man) and the small proportion of the dose absorbed.

Our results do not agree with those of Grady & Stern (1965), although we used the same technique and experimental design, except that a smaller dose of drug was given intramuscularly instead of orally. We found that the concentration in bone was about one-third of that in serum and the half-life of the drug in bone and serum was approxi-

mately the same; no accumulation occurred in bone after repeated intramuscular injections. The results of our animal experiments correspond roughly to those obtained by Holloway, Kahlbaugh & Scott (1963) for bone levels in three patients during continued therapy with 500 to 600 mg lincomycin at 6 hr intervals. These authors found bone levels which were 13.3 to 18.3% of the serum levels.

After a single intramuscular injection of 1.8 g lincomycin we found in one patient bone levels ranging from 5 to 27% of the corresponding serum level.

The results of the animal experiments described here do not confirm the high concentrations of lincomycin in the bones of the rat, as reported by Grady & Stern (1965).

SUMMARY

1. The distribution of the antibiotic lincomycin in serum and bone of the rat was studied after a single intramuscular injection of 20 mg/kg.

2. The concentration of lincomycin was determined by using the agar diffusion technique and *Sarcina lutea* ATCC 9341 as test organism. A serum concentration of about 9.7 μg/ml. was observed 30 min after the injection of the antibiotic; in 6 hr it was not detectable. At all times, the concentration of lincomycin in bone was about one-third of the corresponding concentration in serum.

3. Repeated injections of lincomycin at 12 hr intervals did not cause accumulation in serum or bone.

REFERENCES

GEDDES, A. M., SLEET, R. A. & MCMURDOCH, J. (1964). Lincomycin hydrochloride: clinical and laboratory studies. *Br. med. J.*, **2**, 670–672.

GRADY, J. E. & STERN, K. F. (1965). Penetration of lincomycin into bone. *Interscience Conference on Antimicrobial Agents and Chemotherapy*, 201–205.

GRONDIN, C., ST. MARTIN, M. & POTVIN, A. (1965). Lincomycin and staphylococcal infections: a clinical study of 18 cases. *Can. med. Ass. J.*, **92**, 1062–1065.

HOLLOWAY, W. J., KAHLBAUGH, R. A. & SCOTT, E. G. (1963). Lincomycin: a clinical study. *Interscience Conference on Antimicrobial Agents and Chemotherapy*, 200–203.

HOLLOWAY, W. J. & SCOTT, E. G. (1965). Clinical experience with lincomycin. *Am. J. med. Sci.*, **249**, 691–695.

KAPLAN, K., CHEW, W. H. & WEINSTEIN, L. (1965). Microbiological, pharmacological and clinical studies of lincomycin. *Am. J. med. Sci.*, **250**, 137–146.

MA, P., LIM, M. & NODINE, J. H. (1963). Human pharmacological studies of lincomycin, a new antibiotic for gram-positive organisms. *Interscience Conference on Antimicrobial Agents and Chemotherapy*, 183–188.

MCLEOD, A. J., ROSS, H. B., OZERE, R. L., DIGOUT, G. & VAN ROOYEN, C. E. (1964). Lincomycin: A new antibiotic active against staphylococci and other gram-positive cocci. *Can. med. Ass. J.*, **91**, 1056–1060.

MEYER, C. E. & LEWIS, CH. (1963). Absorption and fate of lincomycin in the rat. *Interscience Conference on Antimicrobial Agents and Chemotherapy*, 169–175.

MCMILLAN, N. L., MCRAE, R. K. & MCDOUGALL, A. (1967). Lincomycin in the treatment of osteomyelitis. *Practitioner*, **198**, 390–395.