

## EFFECTS OF CLOXACILLIN, DOXYCYCLINE, FUSIDIC ACID AND LINCOMYCIN ON THE MECHANICAL PROPERTIES OF BONE AND SKIN IN YOUNG RATS

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The influence of cloxacillin, doxycycline, fusidic acid and lincomycin on the mechanical properties of bone and skin in young rats was examined. The concentrations of the antibiotics in plasma corresponded to therapeutic levels in man. After 14 days of medication the weights of the rats receiving cloxacillin or doxycycline were significantly less when compared with the controls. The doxycycline, the fusidic acid and the lincomycin treated rats had reduced longitudinal growth of femur and reduced tensile strength of intact skin. No differences between any of the antibiotic groups and the control group were found in the tensile strength of incisional skin wounds or in the mechanical properties of the femur and tibia.

*Key words:* bones; cloxacillin; doxycycline; fusidic acid; lincomycin; mechanical properties; skin

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Ideally, antibiotics should be toxic to the bacteria and harmless to the host organism. Some antibiotics such as the tetracyclines, may, however, also impair mammalian cells (Cohlman et al. 1963, Gudmundson 1971, Vazquez 1974). In a previous study we found that oxytetracycline caused reduced bone growth and reduced mechanical strength of both bone and skin in young rats (Engesæter & Skar 1978).

The purpose of the present investigation was to study the influence of four other antibiotics (cloxacillin, doxycycline, fusidic acid and lincomycin) on: Weight gain, longitudinal bone growth, mechanical properties of bone, intact skin and wound healing.

### MATERIALS AND METHODS

Experimental animals were outbred male Wistar/Af/Han/Mol SPF rats, initially weighing

39-44 g. They were kept five in each cage and fed water and Norwegian standard diet for rats *ad libitum*. The animals were divided into five weight-matched groups of ten rats. Group 1 received cloxacillin (Ekvacillin® ad inj., Astra), group 2 doxycycline (Vibramycin® ad inj., Pfizer), group 3 fusidic acid (Fucidin® oral susp., Leo), group 4 lincomycin (Lincocin® ad inj., Upjohn) and group 5 (control) isotonic sodium chloride solution. The antibiotics were given in 0.5 ml of water as intraperitoneal injections every 12 hours for 14 days. Fusidic acid, however, had to be administered by stomach tube as intraperitoneal injections in a pilot study gave massive abdominal adhesions.

The daily amount of antibiotic given was constant throughout the experiment, but as the rats grew rapidly the doses in mg/kg/day were higher initially than at the end. The doses in the middle of medication are given in Table 1.

The concentrations in plasma of cloxacillin, doxycycline and lincomycin were determined by the paper disc method of AB-biodisk (Stockholm, Sweden) (Jalling et al. 1972). Regarding fusidic acid, no commercial standard disc-series was

available. However, a method for determination of the concentration of fusidic acid in rat plasma was obtained from Løvens kemiske Fabrik (Copenhagen, Denmark).

On the first day of medication a 3.5 cm long skin incision was made on the left side of the back of the rats. The wound was closed with three interrupted stitches as previously described (Engesaeter & Skar 1978). The animals were killed with ether after 14 days of medication. Immediately after death the femora and the tibia were dissected free. The length of the femur (from the top of the caput to the distal end of the medial condyle) was measured with a sliding callipers (accuracy of  $\pm 0.01$  mm). Until mechanical testing (less than 2 hours) the femora were kept in isotonic saline solution at room temperature (about 20°C). The left femur diaphysis was tested in torsion and the right distal femur metaphysis in bending according to methods described previously (Engesaeter et al. 1978).

For practical reasons the tibiae were frozen ( $-20^{\circ}\text{C}$ ) for 5 days before testing of bending strength. After thawing (to 20°C), and in a wet condition, the proximal half of the right tibia diaphysis was deflected laterally relative to the distal half.

Skin from the left side of the back containing the healing wound and intact skin from the corresponding right side were removed and frozen for 10–11 days. After thawing the tensile strength of both sutured and intact skin specimens was measured as previously described (Engesaeter & Skar 1978).

In addition to these 50 rats described, 10 animals were killed at the start of the experiment to obtain origin values for femur length, tensile

strength of intact skin and bending strength of the tibia diaphysis.

The median with 25- and 75- fractiles was used to express the average and the dispersion of the measured values. Statistical significance was evaluated by the Wilcoxon test for two samples (one-tailed test) and differences were considered significant if  $P \leq 0.05$  (Diem & Lentner 1975).

## RESULTS

In the middle of the experiment plasma concentrations of the antibiotics were measured 1, 2, 6 and 12 hours after medication (Table 1).

All animals grew rapidly during the experiment (Figure 1), but the cloxacillin and the doxycycline treated rats had, at the end of the medication period, significantly lower body weights (2 and 7 per cent, respectively) than the control rats.

The influence of the antibiotics on longitudinal growth of the femur is illustrated in Figure 2. As compared with controls, the femurs of animals receiving doxycycline, fusidic acid or lincomycin were significantly shorter (1–2 per cent).

No significant differences could, however, be detected in the mechanical properties of the bones from any of the antibiotic treated groups when compared with the controls,

Table 1. Doses and plasma concentrations of the antibiotics on the 7th day of medication. The concentrations were measured in five rats in each group. (Median with 25- and 75-fractiles)

Antibiotic	Dose (mg/kg/12 h)	Plasma concentration ( $\mu\text{g/ml}$ ) at varying intervals after administration			
		1 h	2 h	6 h	12 h
Cloxacillin	248	13 (10–18.5)	<2.0	<2.0	<2.0
Doxycycline	25.5	5.0 (4.5–5.3)	4.5 (4.0–4.6)	2.0 (1.8–2.1)	1.3 (1.2–1.3)
Fusidic acid	238	2.8 (2.8–3.1)	2.0 (1.9–2.2)	1.2 (1.0–3.8)	0.3 (0.2–0.3)
Lincomycin	95	8 (4–8)	<1.0	<1.0	<1.0

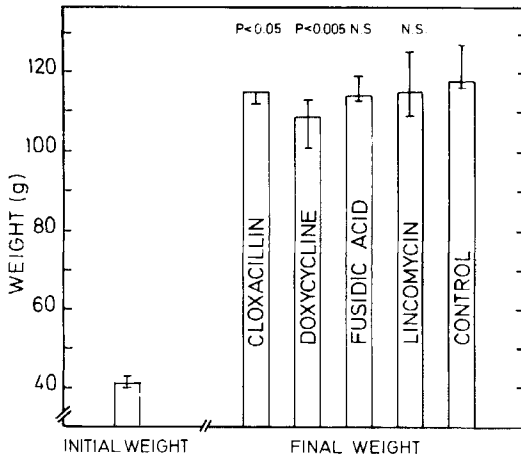


Figure 1. Initial weight and weight after 14 days of medication. (Median with 25- and 75-fractiles).

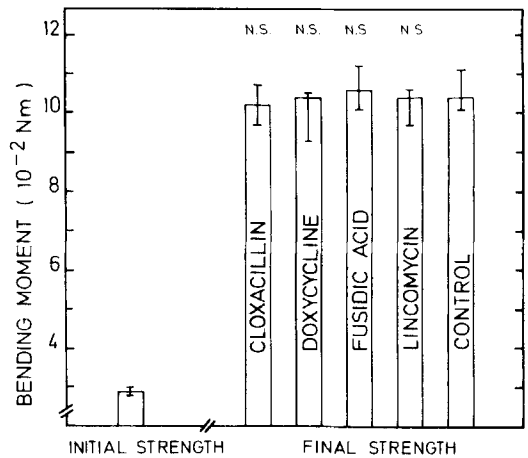


Figure 3. Bending moment necessary to produce fracture in the tibia diaphysis at the beginning of the experiment and after 14 days of medication. (Median with 25- and 75-fractiles).

neither in torsion of the left femur diaphysis, nor in bending of the right distal femur diaphysis or of the right tibia diaphysis (Figure 3).

Testing of the skin wounds revealed no significant tensile strength difference between the antibiotic treated rats and the controls. Tensile strength of intact skin from the doxycycline, the fusidic acid or the lincomycin rats was, however, significantly reduced

compared with skin from controls (22, 23 and 19 per cent, respectively) (Figure 4).

## DISCUSSION

The main features of the present study in young rats are that cloxacillin and doxy-

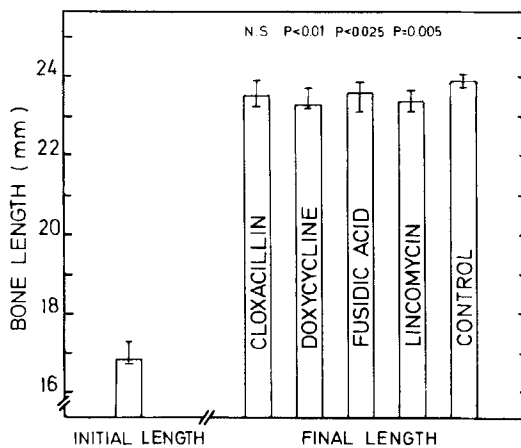


Figure 2. Initial length of right femur and length after 14 days of medication. (Median with 25- and 75-fractiles).

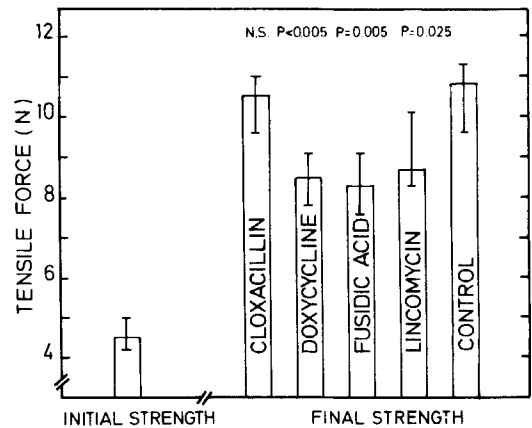


Figure 4. Tensile force necessary to pull apart the intact skin specimens at the beginning of the experiment and after 14 days of medication. (Median with 25- and 75-fractiles).

cycline caused reduced weight gain, and that doxycycline, fusidic acid and lincomycin caused both a reduced longitudinal growth of the femur and a reduced tensile strength of intact skin. No influence of any of these antibiotics could be detected on the mechanical properties of bones or skin wounds.

The doses of antibiotics given, expressed as mg/kg/day, are 2–12 times higher than the recommended maximal human doses (Goodman & Gilman 1975, Otten et al. 1975). However, the plasma concentrations, which are more important, compared well with therapeutic levels in man (Otten et al. 1975).

The observed reduced weight gain in the rats receiving cloxacillin or doxycycline could be caused by an intestinal dysfunction, due, for example, to disturbed intestinal flora or to peritonitis following the intraperitoneal injections. The latter seems, however, less likely as adhesions of the intestines were only found in a few of the doxycycline treated rats.

Rats receiving doxycycline, fusidic acid or lincomycin had shorter femora and a lower tensile strength of intact skin than the controls. These drugs exert their antimicrobial effect through inhibition of the protein synthesis, which may indicate a possible explanation for our observations. In accordance with this, doxycycline and fusidic acid are known to influence the protein synthesis in mammalian cells (Morgan & Ribush 1972, Vazquez 1974, Wynn 1965). Lincomycin, however, is supposed to inhibit the protein synthesis only in bacterial ribosomes (Vazquez 1974).

A possible explanation for the mechanical impairments observed in the present study and in the preceding oxytetracycline experiment may be an inhibition of the cross-linking of collagen (Bailey et al. 1974). The enzyme lysyl oxidase is essential in the first step of cross-link formation and it depends on copper as a co-factor (Siegel et al. 1970). Doxycycline and oxytetracycline are both known to have chelator properties and may inhibit the enzyme by binding copper (Lancet 1978).

In humans, it has been proposed that fusidic acid promotes wound healing (Taylor & Bloor 1962). This effect was ascribed to a proposed anabolic effect of the antibiotic. However, in an experiment with rats Calnan & Fry (1962) could not find any effect on wound healing with 90 mg fusidic acid per os/kg/day (plasma concentrations were not measured). This finding is consistent with our results.

The present study and the previous oxytetracycline study indicate that several antibiotics may impair the mechanical properties of bone or skin in rats. The mechanisms responsible for these effects remain unclear. Collagen is, however, the principal protein in both bone and skin and as this protein is responsible for a major part of the strength in both tissues, it seems worthwhile to elucidate further the effects of these antibiotics on collagen.

## REFERENCES

- Bailey, A. J., Robins, S. P. & Balian, G. (1974) Biological significance of the intermolecular crosslinks of collagen. *Nature (Lond.)* **251**, 105–109.
- Calnan, J. & Fry, H. J. H. (1962) Wound healing in rats with the antibiotic Fucidin. *Brit. J. Pharmacol.* **19**, 321–323.
- Cohlan, S. Q., Bevelander, G. & Tiamsic, T. (1963) Growth inhibition of prematures receiving tetracycline. *Amer. J. Dis. Child.* **105**, 453–461.
- Diem, K. & Lentner, C. (eds.) (1975) *Documenta Geigy. Scientific tables*. 7th ed. pp. 810, Ciba-Geigy Ltd., Basle.
- Engesaeter, L. B., Ekeland, A. & Langeland, N. (1978) Methods for testing the mechanical properties of the rat femur. *Acta orthop. scand.* **49**, 512–518.
- Engesaeter, L. B. & Skar, A. G. (1978) Effects of oxytetracycline on the mechanical properties of bone and skin in young rats. *Acta orthop. scand.* **49**, 529–534.
- Goodman, L. S. & Gilman, A. (eds.) (1975) *The pharmacological basis of therapeutics*. 5th ed. pp. 1704, Macmillan Publ. Co., New York.
- Gudmundson, C. (1971) Oxytetracycline-induced fragility of growing bones. An experimental study in rats. *Clin. Orthop.* **77**, 284–289.
- Jalling, B., Malmberg, A.-S., Lindman, A. &

- Boréus, L. O. (1972) Evaluation of a micro-method for determination of antibiotic concentrations in plasma. *Europ. J. clin. Pharmacol.* **4**, 150–157.
- Lancet* (1978) Collagen in health and disease. *Lancet* **i**, 1077–1079.
- Morgan, T. & Ribush, N. (1972) The effect of oxytetracycline and doxycycline on protein metabolism. *Med. J. Aust.* **1**, 55–58.
- Otten, H., Plempel, M. & Siegenthaler, W. (eds.) (1975) *Antibiotika-Fibel*. 4th ed. pp. 1039, Georg Thieme Verlag, Stuttgart.
- Siegel, R. C., Pinnell, S. R. & Martin, G. R. (1970) Cross-linking of collagen and elastin. Properties of lysyl oxidase. *Biochemistry* **9**, 4486–4492.
- Taylor, G. & Bloor, K. (1962) Antistaphylococcal activity of Fucidin. *Lancet* **i**, 935–937.
- Vazquez, D. (1974) Inhibitors of protein synthesis. *FEBS Letters* **40** (Suppl.), 63–84.
- Wynn, V. (1965) Metabolic effects of the steroid antibiotic fusidic acid. *Brit. med. J.* **1**, 1400–1404.

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